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Factors Influencing the Response of Broiler Chicken to Glycine Supplements in Low Crude Protein Diets

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ABBREVIATIONS

Beneath abbreviations for the units defined by the international system of units and the symbols for chemical elements of the periodic table of elements the following abbreviations were used (with the exception of abbreviations used in chapter 5):

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
Ala	alanine
AME_N	apparent metabolizable energy (N-corrected)
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine triphosphate
BW	body weight
CoA	Coenzyme A
CP	crude protein
Cys	cysteine
DEB	dietary electrolyte balance
DM	dry matter
EC	Enzyme Commission
G:F	gain per feed (feed efficiency)
GfE	Gesellschaft für Ernährungsphysiologie
Gln	glutamine
Glu	glutamic acid
Gly	glycine
Gly_{equi}	glycine + glycine equivalent of serine
His	histidine
Ile	isoleucine
Leu	leucine
Lys	lysine
Met	methionine
NRC	National Research Council
pH	pondus hydrogenii
Phe	phenylalanine
Pro	proline
Ser	serine
Thr	threonine
Trp	tryptophan
TSAA	total sulfur amino acids
Tyr	tyrosine
Val	valine

1 General Introduction

The increasing demand for meat and other animal products along with the global limitation arable land for cropping results in an expected shortage of protein-rich feedstuff. Limited arable land to produce crops for food and feed purposes constrains supply, which, in turn, has an impact on prices. The consequences of increased prices have been shown to especially affect the affordability of food in developing countries (Fazeni and Steinmüller, 2011). Furthermore, the excretion of nitrogenous compounds has negative effects on the environment because the excreted nitrogen (N) is dispersed into environmental water, soil and air. N in manure can be applied reasonably for fertilizing agricultural land, but in too high amounts, this practice risks N leakage into the groundwater (Verstegen and Jongbloed, 2003). Ammonia emissions from livestock enterprises have been associated with a number of environmentally damaging effects, which include soil acidification, eutrophication, formation of fine particulates, and secondary emissions of nitrous oxide (Martínez-Lagos et al., 2013). In addition, ammonia emission affects human and animal health as well as the acceptance of livestock farming by the public due to unpleasant odors (Aneja et al., 2009). Ambient temperature and manure characteristics like pH and moisture have an influence on ammonia emissions, but excretion of N by livestock is the biggest determinant (Liu et al., 2007; Namroud et al., 2010).

Excretion of N in parts is inevitable because of characteristics of the feed, such as digestibility of nitrogenous nutrients, or due to metabolic processes leading to the formation of substances that are subjected to urinary excretion (Berg et al., 2007; Rodehutscord, 2008). However, reduction of N excretion by using highly digestible ingredients and adjusting the supply of nitrogenous nutrients to the requirement of the animal is accomplishable (Baker, 2009).

Experiments, however, show that low crude protein (CP) diets may have undesirable effects on the performance of farm animals and carcass quality, even though the requirement of essential amino acids (AA) was met. At present, there is a substantial lack of knowledge about the requirement of nitrogenous nutrients except for essential AA and the influence of these nutrients on animal physiology. Various explanatory approaches are being discussed as potential reasons for undesirable effects of low CP diets. But neither are their physiological backgrounds entirely clarified nor could selective measures fully overcome the adverse effects.

This doctoral thesis aims to increase the understanding of the effects of reduced CP concentrations in broiler feed and hence to permit further reduction of CP concentrations

without reducing animal performance. The focus of this work is to provide knowledge about factors influencing the response of broilers to dietary glycine (Gly) and serine (Ser) because a deficiency of these nonessential AA is considered as one reason for undesirable effects of low CP diets. A different supply of Gly and Ser is linked to other explanatory approaches that are discussed as factors limiting the potential reduction of CP concentrations in feed. The effect of the dietary concentrations of threonine (Thr) and choline, the relevance of the dietary supply of cysteine (Cys), and the effect of the supply of AA from peptides and free AA will be addressed as linked explanatory approaches.

2 Literature Review

Several explanatory approaches are being discussed as potential strategies to overcome the negative consequences on performance of lowering CP concentrations in poultry feed. This work predominantly focuses on factors influencing the response to Gly and Ser in broiler feed because a deficiency of those AA is a potential reason for reduced growth performance in low CP diets. However, since the approaches are linked with each other, an attempt to isolate one approach would be too simplistic. Therefore, this literature review will first describe approaches of main relevance and then focus on the role of Gly and Ser in avian species.

2.1 Approaches of lowering crude protein concentrations in broiler diets

2.1.1 Different optimal ratio of essential amino acids

It is often discussed that the optimal ratio of essential AA might differ between standard and low CP diets and, therefore, could represent a possible reason for diminished growth when low CP diets are fed to broilers. Low CP diets fortified with free AA to raise the essential AA level of low CP diets to 100% (Deschepper and deGroote, 1995; Kobayashi et al., 2013; Pinchasov et al., 1990), 110% (Jiang et al., 2005; Waldroup et al., 2005), or up to 145% (Bregendahl et al., 2002), based on the recommendations of the National Research Council (NRC, 1984) or NRC (1994) failed to achieve the growth performance observed with standard CP diets. In other studies, combinations of some essential AA (mostly Thr, tryptophan (Trp), arginine (Arg), isoleucine (Ile) and lysine (Lys)) were varied with the result of partially increasing growth performance but without fully overcoming the impaired growth induced by a low CP diet (Fancher and Jensen, 1989a,b; Hussein et al., 2001; Namroud et al., 2008, 2010; Si et al., 2004b,c).

When individual essential AA in low CP diets were maintained to match the corresponding levels of standard CP diets, the probability that single essential AA limit the growth performance of broilers in low CP diets appears unlikely (Awad et al., 2014; Dean et al., 2006; Hurwitz et al., 1998). Higher growth after addition of essential AA might partly be explained by higher fat accretion as a consequence of an increased amount of oxidized AA. This can be caused by a surplus of essential AA above the requirement or different utilization of peptide-bound and free AA (see chapter 2.1.4) in those studies. The interactive effects of some essential

AA with nutrients reduced along with CP reduction, such as nonessential AA, may also have led to different growth.

2.1.2 Nonspecific nonessential amino acids

In the literature, the effect of nonspecific nonessential AA was evaluated by varying either the ratio of the sum of the concentrations of essential AA to the sum of the concentrations of nonessential AA or by altering the sum of the concentrations of nonessential AA. When added to low CP diets, mixtures of free aspartic acid (Asp) and glutamic acid (Glu) (Bregendahl et al., 2002; Leclercq et al., 1994) and a mixture of free Asp, Glu and alanine (Ala) (Nieß et al., 2003) had no effect on growth performance. However, mixtures of free Glu and Gly (Deschepper and deGroote, 1995; Namroud et al., 2008; 2010) and of tyrosine (Tyr) and Ser (Thornton et al., 2006) increased performance but could not achieve the growth performance of diets with standard CP concentrations.

Consideration of nonspecific nonessential AA is probably not sufficient because an unlimited interconversion of nonessential AA is necessary. The results of the aforementioned studies suggest that adequate supply of nonessential AA is important for prevention of a decline in the growth performance of broilers that are fed diets with low CP concentrations, but specific nonessential AA have to be considered.

2.1.3 Specific nonessential amino acids

Studies aiming to increase nonessential AA-N by adding Glu failed to prevent growth depression caused by low CP diets (among others Hussein et al., 2001; Kerr and Kidd, 1999; Pinchasov et al., 1990). Corzo et al. (2005) and Dean et al. (2006) found that diets with 18 and 16.2% CP, respectively, supplemented with Gly to the level of 22% CP control diets caused no difference in growth and feed efficiency compared to the respective control diet. In both studies, Asp, Glu, Ala, and proline (Pro) failed to overcome the negative effects of the low CP diets. Parr and Summers (1991) reported no differences in growth between a diet with 20% CP supplemented with Gly and a diet with 23% CP, and additionally disproved similar effects of Glu, Ala, and Asp. Other studies showed a growth-promoting effect of Gly added to low CP diets (among others Corzo et al., 2004; Jiang et al., 2001; Schutte et al., 1997), whereby the extent of the growth-promoting effect was highly variable. This can potentially be attributed to the heterogeneity of dietary characteristics influencing the response to Gly in these studies. The

physiological background of factors that possibly influence the response to dietary Gly is described in chapters 2.2.1 to 2.2.3.

The potential of Gly to increase growth has been known for decades (Almquist et al., 1940; Almquist and Mecchi, 1940). Since publication of the study of Dean et al. (2006), it is broadly accepted that a deficiency of dietary Gly limits the possibility to reduce the CP concentration in broiler diets and that Gly is the first-limiting nonessential AA (Ospina-Rojas et al., 2012). Waguespack et al. (2009a) described Gly as the fourth-limiting of all proteinogenic AA after methionine (Met), Lys, and Thr in a diet based on corn and soybean meal for broilers from 1 to 18 days post-hatch. Ospina-Rojas et al. (2014) described valine (Val) and Gly as equally limiting after Met, Lys, and Thr in a diet based on corn and soybean meal for broilers from 1 to 21 days post-hatch.

After consideration of recent literature, the next-limiting nonessential AA after Gly is hard to derive. In the study of Dean et al. (2006), addition of Glu and Ala to a low CP mixture elevated feed efficiency to the level of a diet with 22.2% CP, but average daily gain (ADG) remained below the level of the control diet. Both ADG and feed efficiency (G:F) of the treatments with Asp and Pro added to the low CP mixture were below the level of the positive control diet. This might be an indication that either Glu or Ala is the next-limiting nonessential AA after Gly. Pro was found to be a limiting AA because growth and feed efficiency was increased when free L-Pro was added to Pro-free purified diets (Graber and Baker, 1973; Wu et al., 2011). Therefore, the dietary concentration of Pro is likely to become relevant when the CP concentration of diets is further decreased but no statement can be made whether Pro is the next-limiting nonessential AA after Gly.

2.1.4 Utilization of free amino acids compared to peptide-bound amino acids

Different utilization of supplemented free AA compared to peptide-bound AA, synonymously termed “AA from intact protein”, in the metabolism of animals has often been suspected if free AA were used in diet formulation (Jensen, 1991; Surisdiarto and Farrell, 1991; Namroud et al., 2008). Differences are conceivable because di- and tripeptides, and free AA are absorbed differently. Free AA are absorbed into the enterocytes across the brush border membrane via group-specific transport systems, whereas di- and tripeptides are transported across the brush border membrane by intestinal peptide transporters (Krehbiel and Matthews, 2003). Cytosolic peptidases rapidly hydrolyze most of the di- and tripeptides entering the enterocytes and generate free AA. The basolateral membrane of the enterocytes possesses transport systems responsible for the exit of free AA into the systemic circulation. In addition,

unhydrolyzed peptides consisting of mainly two or three AA can be transported intact across the basolateral membrane. This route, however, contributes little to the total AA absorption (Brandsch and Brandsch, 2003). Protein digestion products that are absorbed into the enterocytes are predominantly di- and tripeptides (Krehbiel and Matthews, 2003). If an increased amount of free AA appears in the small intestine due to the inclusion of free AA in diet the formulation, the relative contribution of AA transport systems and peptide transporters to the total AA absorption does change. Several studies found that free AA are absorbed faster than peptides in both mammalian and avian species (Boza et al., 2000; Hansen et al., 1993; Li et al., 1999; Morales et al., 2013). Maenz and Engele-Schaan (1996) found that most of the free L-Met provided disappeared in the anterior section of the small intestine. According to Wu (2009), an imbalance between AA in the systemic circulation as a consequence of faster passage of free AA through the intestinal wall might occur and lead to an increased amount of certain AA catabolized in the enterocytes. Those catabolized AA are then not available for protein synthesis, thus reducing net utilization compared to peptide-bound AA. A reduced oxidative loss after an adaption period of three weeks in rats was interpreted as the capacity to adapt to free AA in diets, with the gastro-intestinal tract being the most likely site for this adaptation (Nolles et al., 2009).

Studies investigating different proportions of peptides and free AA without basically varying dietary AA concentrations in the feed of farm animals are sparse. In pigs, replacement of 144 g/kg casein by the same composition and amount of essential AA in diets did not show significant differences in prececal AA digestibility, average daily feed intake (ADFI), G:F, ADG, and N efficiency (Officer et al., 1997). Another study by the same working group found similar prececal digestibility of AA and ADFI, but significantly lower G:F and, therefore, ADG of pigs fed a mixture of free AA instead of casein (Officer et al., 1998). The N efficiency of the animals receiving free AA was lower and interpreted as an increased proportion of maintenance as a result of the lower growth rate. It is not clear what the reasons for these differences in the results of the two studies were. In both studies, the ratio of the concentration of the sum of essential AA to the concentration of the sum of all AA was maintained at 46% by addition of a mixture of free L-Glu, Gly and L-Pro. Nevertheless, the concentration of one or more nonessential AA might have been too low in the study by Officer et al. (1998).

Similar studies for poultry are not available. Potential differences in N efficiency between free and peptide-bound AA are possibly less pronounced in poultry because the crop portions the feed delivered to the subsequent digestive tract and thereby may mute peaks of certain AA in the systemic circulation.

2.1.5 Acid-base balance

Acid-base homeostasis refers to the endeavor of an animal to maintain a constant intra- and extracellular proton concentration and, thus, pH value. Failure to maintain the internal pH within narrow limits has detrimental effects on the physiology of animals. In healthy animals, increased generation of acids or bases is coupled with physiological adaptations that re-establish acid-base homeostasis. Amino acid metabolism and other nutritional factors influence, and are influenced by, the acid-base balance of an animal (Patience, 1990).

The effect of oxidation on acid-base balance differs among types of AA. Oxidation of neutral AA has no effect on acid-base status, whereas oxidation of dicarboxylic AA causes metabolic alkalosis, while oxidation of dibasic AA and sulfur-containing AA results in metabolic acidosis. Oxidation of phosphorylated AA like phosphoserine causes metabolic acidosis as well (Mackenzie, 1986). In case of Met, two moles of H^+ are generated per mole of oxidized AA (Lemann and Relman, 1959). The prediction of acidogenicity or alkalinogenicity of AA oxidation associated with a particular diet is difficult because it depends on the balance of AA being oxidized, rather than the total AA concentration (Patience, 1990).

If the acid-base balance is disturbed, both AA and proteins are affected. In acute acidotic state, an increased degradation of AA along with visually detected signs of protein deficiency disorders has been observed in humans (Young, 1991). May et al. (1987b) stated that in the presence of acute acidosis, alterations in the metabolism of individual AA are mainly due to altered enzyme activity because enzymes are sensitive to changes in the pH value.

In case of metabolic acidosis in rats, protein degradation was increased by stimulation of tissue proteolysis, whereas protein synthesis was unaffected (Hara et al., 1987; May et al., 1987a). Secretion of hydrogen carbonate ions to the duodenal lumen and blood was found to be an important regulator for the pH value in the intestinal lumen content (Sjöblom and Nylander, 2007) and a main regulator for extra- and intracellular pH (Tresguerres et al., 2010). Dietary administration of sodium hydrogen carbonate to rats in acidotic state was found to overcome proteolysis (May et al., 1987a).

Free AA represent an acid source if supplied as their hydrochloride salts. In case of Lys, which is the most commonly used free AA in animal diets, each g of free Lys·HCl contributes 7 mEq of acid per kg of diet (Patience, 1990). This effect is enforced if, in the course of using AA hydrochlorides, vegetable protein sources with high K^+ concentrations, such as soybean meal or canola meal, or high Na^+ concentrations, such as canola meal, as contributors of alkalinogenicity are reduced (Aftab et al., 2006; Khajali and Slominski, 2012). Other feed components, such as choline chloride, can also alter the acid-base balance (Patience, 1990).

Electrolytes in diets are considered to have an effect on the acid-base balance in animals (Ahmad and Sarwar, 2006) because an excess of cations in diets yields an equivalent excess of metabolizable anions over cations, which represents a contribution of alkaline material. Conversely, in case of relative excess of mineral anions, the associated anions would provide an acid load (Patience, 1990). This influence of electrolytes in feed on the cation-anion balance and, thus, the acid-base balance of animals depends on the binding form, active homeostasis, interactions between electrolytes, and their properties as monovalent or divalent ions (Hooge, 1995; Gorman and Balnave, 1994).

In order to estimate the potential of electrolytes in feed to affect the cation-anion balance in animals, the dietary electrolyte balance (DEB) was introduced. The DEB considers dietary concentrations of Na^+, K^+, and Cl^-, because Na^+ and K^+ can neutralize hydroxyl groups and Cl^- does the same with H^+ ions. Other electrolytes, the effect of binding forms, and effects in the animal organism were disregarded to reduce effort and cost (Ahmad and Sarwar, 2006). However, Patience (1990) questioned the suitability of the DEB for the prediction of acidogenicity or alkalinogenicity due to its many simplifications and restrictions.

Several studies found interactions for ADG, ADFI or G:F between the CP concentration and DEB or the electrolytes included in its calculation (Adekunmisi and Robbins, 1987; Moran and Stilborn, 1996; Murakami et al., 2003), whereas others did not (Cervantes and Jensen, 1986; Fancher and Jensen, 1989a; Han et al., 1992; Martínez-Amezcua et al., 1998, Si et al., 2004a,c). All those studies used pure concentrations of some electrolytes in diets or the DEB as simplified estimates to predict acid-base balance. In addition, most studies used table values for electrolyte concentrations in feedstuffs by what the high variability within types of feedstuffs, e.g. in grain cereals (Rodehutscord et al., 2015), is not considered. This is probably why clear effects cannot be established at present.

Reduction of CP concentration in animal diets is usually linked to different proportions of free AA and other feedstuffs like soybean meal or canola meal in diets. Consequently, the supply of broilers with free AA and electrolytes from feedstuff, and, therefore, potential generation of acids and bases is different in low CP diets compared to standard CP diets. Furthermore, reducing the safety margins in AA supply would result in less AA to be oxidized, which would change the amount of generated acid and base from AA oxidation. The variety of acids or bases producing processes influenced by a reduction of CP concentration in diets make an estimation of net acidogenicity or alkalinogenicity difficult. The animal body reacts with physiological adaptations if the acid-base homeostasis is challenged. These adaptations would

have to be known for targeted countermeasures if recognized as undesirable for broiler production.

At present, the actual consequences of CP reduction on the acid-base balance are unknown because targeted measurements are difficult. Therefore, success in overcoming the negative consequences of lower CP concentrations in broiler feed by selectively influencing the acid-base balance cannot be expected in the foreseeable future. A more promising approach seems to be further optimization of AA concentrations, which might indirectly also optimize the acid-base balance.

2.2 Glycine and serine

Gly has been first identified by Henri Braconnot under the name "sucre de gélatine" in 1820 (Labrude and Becq, 2003). The name "glycine" was derived later from the Greek word "glykys" meaning sweet because it was found to be as sweet as glucose (Wang et al., 2013). The exact composition was determined in 1846 and its structure was first described in 1857. Gly is the simplest AA and has no D- or L-configuration because a single hydrogen atom is attached to the α-C-atom where a side chain is attached for most other AA (Wu, 2013).

Ser was first isolated from sericin by Emil Cramer in 1865 (Cramer, 1865). Its structure was established in 1902. Since sericin was mainly found in silk protein at that time, the name Ser was derived from the Latin word "sericum", which means silk (Belitz et al., 2008).

2.2.1 Endogenous synthesis of glycine and serine

In coaction with tetrahydrofolic acid, Gly can be metabolized from Ser catalyzed by the enzyme serine hydroxymethyl transferase (EC 2.1.2.1) and by the splitting of the hydroxmethyl group of Ser. This reaction can be reversed by adding CH_3 from tetrahydrofolic acid (Velíšek and Cejpek, 2006). Serine hydroxymethyl transferase is present in both the cytoplasm and the mitochondria in the cell. The mitochondrial enzyme is ubiquitous in most cell types, whereas the cytosolic enzyme occurs primarily in the liver and kidneys (Wang et al., 2013). For poultry, it is generally assumed that the interconversion of Gly and Ser is not limited in metabolism (Akrabawi and Kratzer, 1968; Sugahara and Kandatsu, 1976); therefore, they are usually assessed together to determine the physiological value of a diet.

Gly can also be metabolized from Thr via two pathways which mainly occur in the liver. The mitochondrial enzyme threonine dehydrogenase (Enzyme Commission (EC) 1.1.1.103) produces Gly from Thr with 2-amino-3-ketobutyrate as an intermediate metabolic step, which

further reacts to Gly, acetyl-CoA, and aminoacetone (Davis and Austic, 1994). It has been shown in pigs, rats and chickens that this is the major pathway which accounts for about 80% of Thr degradation (Ballèvre et al., 1990; Davis and Austic, 1994). The cytosolic enzyme threonine aldolase (EC 4.1.2.5) metabolizes Thr to Gly with acetaldehyde as an additional product (Malkin and Greenberg, 1964) and was quantified to contribute 7 to 11% of Thr degradation in humans (Darling et al., 2000).

Choline can be metabolically converted to Gly in a five-step reaction in the liver if L-homocysteine is available (Soloway and Stetten, 1953). Choline is metabolized to betaine aldehyde via the enzymes choline monooxygenase (EC 1.14.15.7), choline oxidase (EC 1.1.3.17), and choline dehydrogenase (EC 1.1.99.1). The enzymes betaine-aldehyde dehydrogenase (EC 1.2.1.8) and choline oxidase (EC 1.1.3.17) form betaine from betaine aldehyde, which further reacts to dimethylglycine via betaine-homocysteine S-methyltransferase (EC 2.1.1.5). Dimethylglycine dehydrogenase (EC 1.5.8.4) forms sarcosine from dimethylglycine. Gly is formed from sarcosine via sarcosine oxidase (EC 1.5.3.1) and sarcosine dehydrogenase (EC 1.5.8.3). These reactions are irreversible (Wang et al., 2013) but choline can also be formed from Ser by another metabolic pathway in a nine-step reaction (Meléndez-Hevia et al., 2009; Stekol et al., 1952).

Glyoxylate in combination with Ala is a further source of Gly. In humans, the enzyme alanine-glyoxylate aminotransferase (EC 2.6.1.44) was found to be quantitatively most important for the transfer of the amino group from Ala to glyoxylate, which is a Gly- and pyruvate-forming process (Thompson and Richardson, 1967). In poultry, alanine-glyoxylate aminotransferase was found in the peroxisomes and mitochondria (Sakuraba et al., 1991). There are two enzymes named alanine-glyoxylate aminotransferase with different biochemical properties. These isoenzymes have the same function but their appearance differs among various mammalian (Wang et al., 2013) and poultry species (Sakuraba et al., 1991). One of these enzymes was found to be prevalent in pigeons, sparrows, geese, and ducks, while the other was prevalent in white leghorns, pheasants and Japanese mannikins (Wang et al., 2013).

Moreover, Gly is produced when carnitine is metabolized from trimethyllysine in a four-step reaction (Meléndez-Hevia et al., 2009). Thereby, Gly is a result when 3-hydroxy-trimethyl-lysine is metabolized to trimethyl-amino-butyraldehyde in the presence of the enzyme hydroxytrimethyllysine aldolase (no EC number assigned).

Ser is metabolized from 3-phosphoglycerate in a four-step reaction. The amino group of glutamate is attached to 3-phosphohydroxypyrovic acid in the presence of phosphoserine

transaminase (EC 2.6.1.52), whereby α-ketoglutarate and phosphoserine is produced. The latter is further hydrolyzed and reacts to Ser (Berg et al., 2007).

In adult humans, a daily Gly production capacity was calculated as 2537 mg from Ser, 142 mg from sarcosine, 88 mg from glyoxylate, and 6 mg in the process of carnitine formation (Meléndez-Hevia et al., 2009). Wang et al. (2013) calculated the daily Gly synthesis in young milk-fed pigs as 81 mg/kg body weight (BW) from dietary Ser, 36 mg/kg BW from choline, 33 mg/kg BW from Thr via threonine dehydrogenase, and 1054 mg/kg BW from unknown substrates or other pathways. These authors assumed that Gly might have been converted from 4-hydroxyproline via 4-hydroxyproline oxidase. This pathway was described by Lowry et al. (1985b) but is not listed in biochemistry or metabolism compilations (Wang et al., 2013). The estimate numbers are based on assumptions after the consideration of many studies in humans and other mammals, and cannot directly be transferred to poultry. However, they give an overview of the relation of quantitative contribution of those pathways for endogenous Gly or Ser synthesis.

2.2.2 Physiological functions of glycine and serine

Proteins incorporating glycine or serine

Like any other proteinogenic AA, Gly and Ser are incorporated in proteins. The total accretion of Gly and Ser in broilers from d 8 to 21 was determined between 7.8 and 11.4 g Gly accretion and between 4.2 and 5.5 g Ser/16 g N accretion (Fatufe et al., 2004; Fatufe and Rodehutscord, 2005). Wu et al. (2013) described the Gly and Ser concentration in the protein of 10-d-old chickens without intestinal lumen contents as 11.5 and 4.5 g/16 g N, respectively. The lack of a side chain of Gly leads to some physical characteristics like size, imparting charge, and hydrophobicity. These features cause a possible accommodation in the hydrophobic interior of proteins, which leads to flexibility in the folding of proteins with a propensity to form helices and causes versatility in the structure of receptor sites as well as flexibility for active sites of enzymes (Hall, 1998; Yan and Sun, 1997). Besides asparagine (Asn), Thr, hydroxyproline and hydroxylysine, Ser is capable of being a binding site between proteins and carbohydrates of glycoproteins (Marshall, 1974).

The proteins richest in Gly are collagen and elastin, where Gly is incorporated at every third position in the primary structure (Meléndez-Hevia et al., 2009). The assembly of the triple helix of collagen has the Gly residue at the interior of the helix, where there is no space for larger side groups than the single hydrogen atom in the side chain of Gly (Wang et al., 2013). In broiler

slaughter processing, low skin strength due to low collagen content as a consequence of low dietary supply with Gly can have economic implications (Christensen et al., 1994).

Quasi-repetitive peptide sequences are present in keratins and intermediate filament proteins like nuclear lamins (Steinert et al., 1991). Keratin, which is rich in both Gly and Ser, consists of a large number of heterogeneous proteins. In avian species, keratin is mainly present in feathers and claws (Busch and Brush, 1979). The feather development of birds fed with diets deficient in Gly is impaired (Fisher et al., 1955; Robel, 1977).

Mucin proteins are rich in Ser and Thr (Lien et al., 1997) because both AA provide attachment sites for the oligosaccharide chains, which have a high proportion in mucins (Montagne et al., 2004). The physiological functions of mucins are described as lubrication of the gut epithelium, protection of the epithelium against acidic conditions and proteases, and a selective diffusion barrier for nutrients. Further microbiota-associated functions are fixation of commensal bacteria, protection of the epithelium from pathogens, and substrate for bacterial fermentation (Montagne et al., 2004). Ospina-Rojas et al. (2013a) found that intestinal mucin secretion of broilers linearly increased with the proportion of dietary Gly+Ser at a low Thr level and reached a plateau at a high Thr level. They stated that as the number of goblet cells in the intestine remained unaffected and that their production was increased because more base substance for mucin synthesis was available.

Processes using metabolization products of glycine or serine

In uricotelic species like birds, ammonia is detoxified and excreted as uric acid, which is the main excretion product of the N metabolism (Kikuchi et al., 2008). The formation of each molecule of uric acid requires one molecule of Gly to build the purine ring when glycinamide ribotide is synthesized from phosphoribosylamine (Bloomfield et al., 1969; Patience, 1990). In addition, protein synthesis and cell proliferation depend on DNA synthesis, which requires Gly to form purines (Wang et al., 2013).

Gly is an integral part of creatine, along with Arg (Bloch and Schoenheimer, 1940). Creatine can either be directly supplied by feed derived from animal products or produced by endogenous synthesis, which occurs in a two-step reaction. The first step is catalyzed by the enzyme L-arginine:glycine amidinotransferase (EC 2.1.4.1). There, L-Arg reacts with Gly to form L-ornithine and guanidino acetic acid. This takes place mainly in the kidneys, the pancreas, and in the liver (Smith and Lewis, 1963). In the second step, guanidino acetic acid is methylated at the amidino group by S-adenosyl-L-methionine to form creatinine in the liver (Bloch, 1946; Michiels et al., 2012). Several studies have shown that creatine concentration in the pectoral

muscle increases when Gly is supplemented to diets (Ngo et al., 1977; Ospina-Rojas et al., 2013b).

Most species cannot synthesize Cys *de novo* but can metabolize Cys from Met (Berg et al., 2007). L-Met is metabolized to L-homocysteine with S-adenosyl-L-methionine and S-adenosyl-L-homocysteine as intermediate steps. L-Ser is required when L,L-cystathionine is formed from L-homocysteine by the action of cystathionine β-synthase (EC 4.3.1.22). L,L-cystathionine further reacts to L-Cys, 2-oxobutyric acid, and ammonia by cystathionine γ-lyase (EC 4.4.1.1) (Velíšek and Cejpek, 2006).

Primary bile salts are synthesized from cholesterol in the liver and then conjugated with either Gly or taurine by the enzyme bile acid-CoA:amino acid N-acyltransferase (EC 2.3.1.65) (Falany et al., 1994). Bile salts operate to promote the digestion and absorption of fats and liposoluble substances like vitamins (Berg et al., 2007). Most of the bile salts are reabsorbed in the small intestine (Meléndez-Hevia et al., 2009). The proportion of bile salts conjugated with Gly or taurine is different between species. In avian species, bile salts are almost exclusively conjugated with taurine (Hofmann et al., 2010). Dietary Gly supplementation was found to increase apparent fat digestibility in broilers (Alzawqari et al., 2010; Ospina-Rojas et al., 2013a) and laying hens (Han and Thacker, 2011), and, consequently, it raised the apparent metabolizable energy concentration of feed (Ospina-Rojas et al., 2013a). This has been interpreted by the authors as a consequence of different levels of bile production in consequence of Gly availability. However, this conclusion appears unlikely because the formation of Gly-conjugated bile salts is particularly low in chicken (Elkin et al., 1990; Hofmann et al., 2010).

Each porphyrin, such as heme, is formed from succinic acid and Gly (Shemin, 1970). The formation of each heme group dissipates eight molecules of Gly (Meléndez-Hevia et al., 2009; Shemin, 1970). Thus, Gly is involved in the formation of heme-containing compounds like myoglobin, hemoglobin or cytochromes (Meléndez-Hevia et al., 2009).

Gly was found to be a neurotransmitter in the central nervous system, thereby mainly occurring in the spinal cord (Aprison and Werman, 1965). The qualification as a neurotransmitter is the release of Gly from the spinal cord after appropriate stimulation, the existence of a mechanism to regulate the transmission after release, specific Gly-sensitive receptors, and other substances antagonizing the action of Gly (Hernandes and Troncone, 2009). There is evidence that also Ser, especially D-Ser, has a regulatory role in the central nervous system (Kleckner and Dingledine, 1988), but those modes of action are less understood (Hernandes and Troncone, 2009).

Palmitoyl-CoA and L-Ser react to 3-ketosphinganine (Brady and Koval, 1954), which is the precursor of ceramide. Ceramide further reacts to glycosylceramids and sphingomyelins, which are ubiquitously distributed in the body, particularly in brain tissues. As membrane components, those compounds have a variety of biological functions including membrane transport, cellular apoptosis, cell adhesion, aging, protein exocytosis, and protein trafficking (Hirabayashi and Furuya, 2008).

2.2.3 Degradation of glycine and serine

Gly is mainly catabolized via decarboxylation and deamination by the mitochondrial Gly cleavage enzyme system, which was found in animals, plants and bacteria (Kikuchi et al., 2008; Lowry et al., 1985a). This system catalyzes a reversible reaction, where Gly and tetrahydrofolate are metabolized to 5,10-methylene-tetrahydrofolate, carbon dioxide, and ammonia in a multistep process that requires four specific proteins (Kikuchi et al., 2008). The activity of the Gly cleavage system in the liver and Gly degradation was shown to increase in the status of metabolic acidosis in rats (Lowry et al., 1985b). Moreover, the Gly cleavage system has also been shown to represent the quantitatively most important pathway of Ser catabolism in several animal species (Kikuchi et al., 2008; Yoshida and Kikuchi, 1973). In uricotelic animals like chicken, the Gly cleavage system supplies one-carbon compounds from Gly degradation for purine synthesis (Kikuchi et al., 2008; Sonne et al., 1946). Ser can also directly be deaminated to pyruvate and ammonia in the presence of serine dehydratase (EC 4.3.1.17) (Berg et al., 2007).

The energetic yield of oxidation was calculated as 0.173 moles of net adenosine triphosphate (ATP) production per g of Gly if Gly is catabolized by the Gly cleavage system. A net ATP production of 0.124 moles per g of Ser was determined (Wu, 2009).

2.2.4 Reference units for glycine and serine and concentrations in feedstuffs

As described in chapter 2.2.1, it is generally assumed that for poultry the metabolic interconversion of Gly and Ser is not limited. Therefore, Gly and Ser are usually assessed simultaneously to determine the physiological value of a diet. Most studies use the sum of the concentrations of both Gly and Ser, usually termed “Gly+Ser” (e.g. NRC, 1994), to capture the analogous effect of these AA. This is a simply applicable reference unit, but does not account for the fact that dietary Ser only has the same effect as Gly on an equimolar basis (Akrabawi and Kratzer, 1968; Sugahara and Kandatsu, 1976). Consequently, Akinde (2014) and Dean et

al. (2006) proposed using Gly equivalents (Gly_{equi}) as a reference unit, which is calculated as the sum of the concentration of Gly and the molar equivalent of the Ser concentration. If possible, Gly_{equi} will be used as the reference unit in this thesis because Gly_{equi} should meet the physiological value of a diet more appropriately than Gly+Ser.

The concentration of Gly_{equi} varies considerably between and within types of feedstuffs (Table 2-1). However, the variation in the proportion of Gly and Ser in CP is low and ranges between 4 and 5 g/16 g N, and Gly_{equi} between 6.5 and 8.0 g/16 g N in most cereals, cereal byproducts, brewery byproducts, and pulses. Compared to those types of feedstuffs the proportion of Gly_{equi} in CP is slightly elevated in some oilseeds and oilseed meals. In milk products the concentration of Ser in CP is approximately the same as in the previously mentioned types of feedstuff. However, the proportion of Gly in CP is lower (1.8 to 1.9 g/16 g N). Variation within animal byproducts is most pronounced. The proportion of Gly, Ser, and Gly_{equi} in CP in feedstuffs based on animal blood is on the same level as in oilseeds and oilseed meals. Fish meal contains low proportions of Gly, Ser, and Gly_{equi} in CP. The proportion of Gly_{equi} in CP is highest in feather meal, meat and bone meal, and meat meal (14.7, 17.7, and 14.8 g/16 g N, respectively), which is due to the high proportion of keratine in feathers (Weiss and Kirchner, 2011) and high Gly proportion in total body protein (Fatufe and Rodehutscord, 2005; Wu et al., 2010).

According to these data, animal byproducts like feather meal, meat meal or meat and bone meal are appropriate to elevate the Gly_{equi} concentration in diets for poultry or other species. However, feeding animal-derived protein to farm animals is generally prohibited in Europe at present (European Commission, 2001), except for fish feeding (European Commission, 2013). Feed additives potentially suitable for elevating the Gly_{equi} concentration in feedstuff are free Gly and L-Ser, which are currently not approved in Europe as well (European Commission, 2014).

Table 2-1. Concentration of Gly_{equi} and proportion of Gly, Ser and Gly_{equi} in the protein of selected feedstuffs relevant for animal feeding (extracted from Evonik, 2010).[1]

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

[1] Gly = glycine, Ser = serine, Gly_{equi} = Gly + Gly equivalent of Ser, DM = dry matter, DDGS = distillers dried grains with solubles

3 Overview and Research Questions of the Included Studies

At the beginning of the work on this doctoral thesis it was decided to focus on the role of Gly and Ser in reduced CP diets. At the time, the potential of dietary Gly and Ser to overcome reduced growth performance in low CP diets had been known but the response to dietary Gly and Ser was inconsistent in literature. Therefore, the studies presented in chapter 5 of this thesis were carried out to provide information about factors influencing the response to Gly and Ser in reduced CP diets of broilers and were selected from all the influencing factors mentioned in chapter 2.

The objectives of the first study (chapter 5.1) were to derive information from existing literature. We hypothesized that the response to dietary Gly and Ser can be quantitatively derived from published studies and that the literature provides further information about dietary characteristics that enable rendering the response to dietary Gly and Ser more precisely.

The second study (chapter 5.2) was conducted to describe variation in response to dietary Gly and Ser at different dietary concentrations of Thr and choline as endogenous precursors of Gly. For Thr, an influence has already been known, whereas for choline no information could be found in the literature. Therefore, the objective of this study was to investigate the extent of interactive effects among Gly and Ser, choline, and Thr in a growth study with broilers.

One objective of the third study (chapter 5.3) was to evaluate whether the growth performance and N utilization of broilers are influenced by substituting peptide-bound AA from soy protein isolate with free AA in diets with low CP concentration due to different proportions of free and peptide-bound AA being present in diets. The other objective of the third study was to investigate whether the proportion of peptide-bound and free AA in diets influences broiler Gly_{equi} requirements.

4 General Discussion

Lowering the CP concentration of broiler diets is an effective tool for decreasing N emission related to meat production and for decreasing costs by more efficient use of protein in feedstuff. Experiments, however, showed that low protein diets may have undesirable effects on the performance of farm animals and carcass quality, even though the requirement of essential AA is met. Various potential reasons have been suggested to explain the undesirable effects of low CP diets (chapter 2.1). Since the importance of Gly_{equi} in poultry nutrition has been recognized, the publication count of studies enlarging knowledge about the effects of Gly and Ser has increased. Published literature indicated that the response to dietary Gly_{equi} was inconsistent. The studies presented in chapter 5 of this thesis were carried out to provide information about factors influencing the response to Gly_{equi} in reduced CP diets of broilers.

Based on the findings from the studies of the present thesis, the effect of factors influencing the response to dietary Gly_{equi} as the main focus of this work are debated in this general discussion. A different utilization of peptide-bound and free AA as another potential reason for undesirable effects of low CP diets will also be discussed. Since only a few factors influencing the response to dietary Gly_{equi} could be targeted in this thesis, further areas of investigation will be presented. The general discussion will also deal with the role of Gly and Ser in recommendations, as well as with perspectives regarding the reduction of CP concentration in broiler diets.

4.1 Error analysis and methodological considerations

4.1.1 Sampling errors

Measurements are inevitably linked with errors. Errors may have occurred when the data for this work were acquired despite best efforts to avoid them. It is reasonable to discuss possible sources of error in order to improve interpretability of the results presented in this work.

Bird weight and feed intake were recorded as response traits in the studies presented in chapter 5.2 and 5.3. Dead birds were weighed at least once daily after discovery. Birds lose weight by moisture loss when perished which is interpreted as lower growth. This inaccuracy is negligible and probably did not considerably influence the results. Feed intake was determined as the difference between feed weight at the beginning and at the end of a time

period for each observation. Some feed pellets might have fallen on the floor of the pens or through the gridded floor of the metabolism cages. If possible, such pellets were collected and assigned to the respective pen or cage. Otherwise, such feed loss was erroneously recorded as consumed feed. The relevance of this error is particularly important at an early age of birds, when the actual feed intake is low. This might have contributed to the absence of a treatment effect in the period between day 1 and 7 post-hatch in the study presented in chapter 5.2. However, whether the results would differ if feed loss had not occurred will remain speculation. For the following age periods and probably also for the period between day 1 and 7 post-hatch the relevance of this error appears low because of the low proportion of potential feed loss and actual feed intake.

In the study presented in chapter 5.3 data based on the quantitative collection of excreta were given. Possible excreta residues at the edge of the cage and on the gridded floor cannot entirely be excluded despite accurate sampling. These residues would lead to an underestimation of feces and urine excretion and their constituents. Since the N balance is calculated as N intake minus N excretion, uncollected excreta lead to an overestimation of the N balance. Due to the low proportion of possible excreta residues in the cages to the total amount of excreta, this error appears rather small.

It was unavoidable that some feed pellets fell through the gridded floor of the metabolism cages, whereby some pellets came in contact with the excreta to be collected as samples. Feed and excreta were separated as accurately as possible, but especially in the case of wet excreta, low amounts of feed might have been mixed with excreta. On principle, N in feed wrongly considered as excreted N decreases the determined N accretion. However, as the amount of feed mixed with excreta must have been small and did not appear to be influenced by treatment, a limitation of the trustworthiness of the results is estimated to be low.

As the meta-analysis presented in chapter 5.1 is based on experimental work conducted by other researchers the accuracy of the entire data sampling process in the single studies is unknown.

4.1.2 Methodological considerations

In contrast to sampling errors, methodological limitations are consequences of conscious decisions after balancing the advantages and disadvantages of several possibilities.

Choice of methods

The meta-analysis presented in chapter 5.1 brings together the results of 11 experiments that have been published previously. The number of treatments per experiment varied from 4 to 31. As the statistical model considered each treatment in the data set as equal, the contribution of single experiments to the results depended on the number of treatments per experiment. This effect could have been compensated by weighing the data lines in the data set by the number of treatments per experiment. As a consequence, studies with a high number of treatments would contribute to the results to a lower extent. Those considerations led to the decision not to statistically weigh the contributions of treatments in any way.

The meta-analysis was based on the results of published experiments conducted by other researchers. Different opinions exist among researchers concerning the declaration of data. Some researchers prefer to declare nutrient concentrations on a dry matter (DM) basis, whereas others declare on a standardized 88% DM basis or state the concentration in the feed as it was fed. The results of the meta-analysis refer to the concentration in the feed as it was fed because this option was chosen in most experiments. However, in some cases, it was not recognizable which DM basis the given nutrient concentrations refer to. Comparisons of the stated nutrient concentrations with the respectively used recommendations suggest that the declarations were based on feed as it was fed and treated as such. Another aspect is that some authors only declared calculated nutrient concentrations. In this case, an unknown uncertainty exists regarding the extent to which the actual nutrient concentrations differed from the declared concentrations. Several authors provided the sum of Met and Cys, while others provided total sulfur AA (TSAA). However, several studies provided TSAA while disregarding sulfur-containing AA, other than Met and Cys. In the meta-analysis, TSAA was used because 7 of the 10 studies used this notation. Despite the notation Met+Cys probably being more correct, TSAA will be used in the following sections of the general discussion in order to avoid confusion with the different notations.

Due to the passage time of feed in the digestive tract the N excretion during a time period is partly a consequence of N intake before this period. For growing animals, this makes accurate values for N accretion in short periods of observation difficult to determine because the feed intake usually increases when animals grow. In addition, the amount of collected excreta might be influenced by the coincidental frequency of the occurrence of excretion in short collection periods. Longer collection periods of four and more days are usually applied (e. g. Bourdillon et al., 1990; Samadi and Liebert, 2006) to disperse these effects over several days. The excreta used to calculate N accretion in the study presented in chapter 5.3 were collected quantitatively

for 24 hours. The decision for this duration was a compromise between measurement accuracy, usage of the animals for other measurements, and workload of the involved persons. The influence of the coincidental frequency of occurrence of excretion is diminished by the high number of replicates per treatment. Consequently, the level of the values for N accretion may not represent the actual N accretion on the respective observation days but the differences between the treatments as the main criterion could be determined to a satisfactory level.

The chemical composition of excreta changes after the excretion because of microbial activity, exposition to the environment, and losses to the environment (Applegate et al., 2008; Nahm, 2003). Microorganisms in the excreta produce, for instance, the enzyme uricase, which enables the conversion of uric acid to allantoin. The latter is further converted to glycolic acid, urea, and ammonia. In addition, microorganisms produce the enzyme urease, which catalyzes the degradation of urea to ammonia and carbon dioxide. Ammonia as a volatile compound can be dispersed into the environment (Nahm, 2003). The N in possibly volatilized ammonia could not be determined as N excretion and, thus, might have led to an overestimation of N accretion. Furthermore, the proportion of uric acid, ammonia, and other nitrogenous compounds in excreta might have changed as a consequence of the processes mentioned above. The literature lacks information about the effects of excreta sampling intervals on the composition of excreta. Sampling intervals of twice daily (e.g. Shafey et al., 2013), once daily (e.g. Macleod, 1997) or longer (e.g. De Paula Dorigam et al., 2014) have been described in the literature. In the study presented in chapter 5.3, excreta were collected in 8-hour intervals because ammonia as a volatile compound in excreta was a targeted response trait. This sampling interval has been chosen by weighing an unknown degree of error against the workload of the involved persons.

Limits of applicability of Gly_{equi} as a reference unit

The generally accepted theory of equal effectiveness of dietary Gly and Ser (Akinde, 2014; NRC, 1994) is based on four studies from the 1960s and 1970s. This section aims to present the conditions under which the equal effectiveness was postulated and in which sense it can be applied in current conditions.

An equal effectiveness of dietary Gly and Ser on a molar basis was described in studies with broilers (Akrabawi and Kratzer, 1968; Baker et al., 1968; Featherston, 1975) and roosters (Sugahara and Kandatsu, 1976). Featherston (1975) described the interconversion of Gly and Ser as not limited in metabolism. In the studies of Akrabawi and Kratzer (1968), Baker et al. (1968) and Featherston (1975) diets including up to 12 g/kg Gly or 16.8 g/kg L-Ser as the equimolar amount of 12 g/kg Gly were evaluated. Featherston (1975) also reported no

difference in ADG, G:F and uric acid excretion of 16.8 g/kg dietary L-Ser and an equimolar mixture containing 6 g/kg Gly and 8.4 g/kg L-Ser. The equal effectiveness was determined with animals that reached 9 to 13 g ADG from day 7 to 14 post-hatch (Akrabawi and Kratzer, 1968; Baker et al., 1968), and 16 to 25 g ADG from day 10 to 18 post-hatch (Featherston, 1975). Thus, the assumption of unlimited interconversion is well-established for these Gly and Ser concentrations and response criteria.

There are two potential limitations of the theory of unrestricted interconversion of Gly and Ser in metabolism that constrain the applicability for today's breeding end products, such as Ross 308. First, a potential maximum extent of this interconversion might have been undetectable because a higher metabolic conversion was unnecessary as a consequence of the low growth rate in the studies by Akrabawi and Kratzer (1968), Baker et al. (1968), and Featherston (1975). Today's Ross 308 end products are stated to reach 43 g ADG in the second week of age (Aviagen, 2014). This difference in growth can be explained by the purified diets used by Akrabawi and Kratzer (1968), Baker et al. (1968) and Featherston (1975), which usually cause lower growth rates, and also by the increased growth potential of current broiler end products compared to older ones. The second limitation is that the required interconversion between Gly and Ser in metabolism might be higher than precipitated by 12 g/kg Gly or 16.8 g/kg Ser in diets. Recent studies (Dean et al., 2006; Powell et al., 2009, 2011; Waguespack et al., 2009b) as well as the findings presented in chapter 5 show that the required dietary Gly_{equi} concentration needed to let broilers express the potential of growth performance possibly exceeds the concentrations evaluated by Akrabawi and Kratzer (1968), Baker et al. (1968), and Featherston (1975). However, these are theoretical considerations and no data are available supporting the view of limited interconversion.

Although the specific requirement for Gly or Ser is unknown, the necessity of a metabolic conversion between Gly and Ser in the currently common diet formulation is assessable. In most vegetable feedstuffs the proportion of Gly in Gly_{equi} ranges from 49 to 66% (Table 4-1). This proportion is generally lower in milk byproducts and higher in some animal byproducts, especially in meat and bone meal. The proportion of Gly in Gly_{equi} accreted per kg BW gain from day 8 to 21 post-hatch was 71% in the studies of Fatufe et al. (2004) and Fatufe and Rodehutscord (2005). Deriving required concentrations of certain AA in feed only from the respective AA concentration in body tissue is difficult because of metabolic processes such as degradation in the enterocytes, utilization of AA to form other AA or utilization of the metabolization products of AA. Assuming an equal accretion of Gly and Ser in the feed for

accretion in the body, the supply of Gly in Gly_{equi} from usual diets is within the range of interconversion between Gly and Ser described in the literature.

Table 4-1. Concentration of Gly, Ser and Gly_{equi} as well as percentages of Gly in Gly_{equi} in selected feedstuffs (extracted from Evonik, 2010).[1]

Feedstuff	Gly (g/kg DM)	Ser (g/kg DM)	Gly_{equi} (g/kg DM)	Gly/Gly_{equi} (%)
Cereal grains				
Barley	4.9	5.1	8.5	57
Corn	3.5	4.3	6.6	53
Durum	5.3	7.3	11.1	53
Oats	4.8	5.7	8.8	54
Rye	4.7	4.5	7.9	59
Triticale	5.3	5.8	9.5	56
Winter wheat	4.7	5.1	8.3	56
Cereal byproducts				
Corn bran	5.0	5.0	8.6	58
Corn gluten feed	10.3	9.4	17.1	61
Wheat bran	9.1	7.5	11.4	63
Wheat gluten feed	27.7	40.1	56.4	49
Brewery byproducts				
DDGS (wheat)	12.6	13.9	22.5	56
Brewer’s dried yeast	19.7	21.9	35.3	56
Pulses				
Field beans	12.0	13.4	21.6	56
Field peas	10.5	11.3	18.5	57
Lupins	16.1	20.0	30.4	53
Oilseeds and meals				
Rapeseed (full fat)	11.0	9.2	17.6	63
Rapeseed meal	19.9	16.6	31.7	63
Soybean meal	22.5	26.7	41.6	54
Sunflower expeller	18.9	13.5	28.5	66
Animal byproducts				
Blood meal	43.0	47.0	76.6	56
Blood plasma protein	30.4	52.1	67.6	45
Feather meal	65.7	90.1	130.1	51
Fish meal	47.1	24.1	64.3	73
Meat and bone meal	73.4	18.9	86.9	84
Meat meal	64.9	27.4	84.5	77

[1] Gly = glycine, Ser = serine, Gly_{equi} = Gly+ Gly equivalent of Ser, DM = dry matter, DDGS = distillers dried grains with solubles

Despite the gaps in knowledge discussed above, the theory of unrestricted interconversion between Gly and Ser still appears applicable for the current diet formulation, therefore, there is justification for applying it in new investigations. For a secure statement, however, a targeted investigation would be necessary where the same dietary concentrations of dietary Gly_{equi} (above 22 g/kg DM) achieved with different concentrations of Gly and Ser are evaluated at a currently common growth rate.

Specification of the requirement

In the study presented in chapter 5.1, distinct recommendations for dietary Gly_{equi} concentrations were not specified. Instead, descriptions of requirement values dependent on the intended performance level of the different analyses were given. This alternative has the advantage that situational economic considerations influencing the nutrient concentration of individual batches with maximum economic return can be taken into account. A disadvantage of this method is an increased responsibility of the person in charge of diet formulation to derive the required information. Economic aspects of optimal dietary concentrations are usually entirely neglected when recommendations are derived in order to be independent of market situations. As operators prefer single requirement values for nutrients, recommendations are usually not given as variable but generally assumed to be 95% of maximum response (Rodehutscord and Pack, 1999). In order to increase comparability to previous studies, the study presented in chapter 5.2 gave examples of requirement values at 95% of maximum response. In addition, requirement values for other percentages of maximum response can be derived from the given data. In the general discussion of this thesis, requirement values are discussed at a level of 95% of maximum response to increase comparability of the findings described in chapter 5 with previous studies. Nonetheless, this discussion provides a basis to deduce requirement values at other levels of maximum response.

4.2 Factors affecting the growth response to dietary Gly_{equi}

The main aim of this work was to describe reasons for different responses to dietary Gly and Ser that were determined in literature. Previous dose-response experiments investigated growth and feed efficiency in experimental periods between day 1 and 22 post-hatch. Comparability of the recommended Gly+Ser or Gly_{equi} values in the studies is difficult because the authors used the concentration at maximum response or defined the requirement as the dietary concentrations that led to certain percentages of maximum response. The studies were reevaluated in own

Table 4-2. Determined concentrations of dietary Gly_{equi} and Gly+Ser needed to achieve 95% of the maximum ADG and G:F response in dose-response studies varying the dietary glycine concentrations.[1,2]

Study	Experiment	Experimental period (d)	Gly_{equi} (g/kg)		Gly+Ser (g/kg)	
			ADG	G:F	ADG	G:F
Corzo et al., 2004		7 to 20	12.9	11.4	15.1	13.6
Dean et al., 2006		1 to 18	17.3	16.6	18.9	18.2
Heger and Pack, 1996	1	5 to 20	11.9	11.4	12.5	12.0
	2	5 to 22	n.d.[3]	11.5	n.d.	11.5
	3	5 to 22	n.d.	12.6	n.d.	12.7
Ngo et al., 1977		11 to 13	19.3	11.7	20.9	13.3
Schutte et al., 1997	1	1 to 14	13.5	13.4	15.9	15.8
	2	2 to 21	14.8	14.1	17.4	16.7
	3	1 to 14	13.4	12.3	15.8	14.7
Waguespack et al. (2009a)			-[4]	-[4]	n.d.	16.5
Waldroup et al., 2005		1 to 21	18.1	>23.6[4]	21.3	>27.3[4]
Minimum			11.9	11.4	12.5	12.0
Maximum			19.3	>23.6[5]	21.3	>27.3[5]
Meta-analysis[5]			16.1	15.8	18.5	18.0

[1] Gly_{equi} = Glycine + glycine equivalent of serine, Gly = glycine, Ser = serine, ADG = average daily gain, G:F = feed efficiency
[2] Own recalculations; values obtained by second-order polynomial regression analyses of the data presented in the respective studies
[3] Not detectable
[4] Could not be calculated because the authors did not give Gly and Ser separately
[5] Above the measured range
[6] Meta-analysis in the study presented in chapter 5.1; the values for Gly+Ser are based on the data set designated G+S11

calculations by fitting the given results to second-order polynomial regressions in an attempt to make the published results comparable. Table 4-2 summarizes the required Gly_{equi} concentrations at 95% of maximum ADG and G:F of the studies, where the regression satisfactorily fitted the given data. The determined concentrations are also given as Gly+Ser to enable comparing the results of these studies to existing recommendations in chapter 4.3.1. In fact, 95% of the maximum response was reached at Gly_{equi} concentrations from 11.9 to 19.3 g/kg and from 11.4 to higher than 23.6 g/kg for ADG and G:F, respectively. This demonstrates that the response to dietary Gly_{equi} is highly inconsistent in published literature. It has been

shown that an adequate supply of Gly_{equi} can overcome negative effects of the low CP diets (Dean et al., 2006). In the meta-analysis presented in chapter 5.1, requirement values of 15.8 and 16.1 g/kg Gly_{equi} were determined to achieve 95% of maximum ADG and G:F, respectively. However, these values resulted from a compilation of 11 experiments published in 10 studies where factors influencing the requirement for Gly_{equi} were not considered in most cases. Given the wide range of response to dietary Gly_{equi} (Table 4-2) an estimation of a certain required dietary Gly_{equi} concentration in low CP diets without affecting growth performance is difficult.

Several factors are discussed as potential influencing factors on the response to dietary Gly_{equi}. The studies presented in chapter 5 deal with some of those possible influencing factors. The following section aims at combining those findings and points out further aspects for future investigation.

4.2.1 Evaluation based on present findings

Conversion of methionine to cysteine

Powell et al. (2011) were the first to describe that the increasing impact of Gly and Ser on G:F in parts can be explained by the conversion of Met to Cys, for which Ser is required (chapter 2.2.2). In their study an increased G:F in response to addition of Gly to a diet adequate in TSAA but deficient in Cys was found. Addition of Cys above the recommendations of the NRC (1994) reduced the growth performance, thus increasing the effect of supplemented Gly, whereas inclusion of Met showed no increase in performance. Other studies describing such an effect in poultry or other farm species were not published.

The effect described by Powell et al. (2011) was confirmed in the meta-analysis presented in chapter 5.1. In most of the studies used for the meta-analysis, dietary concentrations of essential AA, including TSAA, were in accordance with or exceeded the recommendations of the NRC (1994) (arithmetic mean 8.7 g/kg TSAA, standard deviation 0.7 g/kg in 125 treatments). In the considered studies, the recommendations of TSAA were met by addition of free DL-Met and not Cys. One aspect found in the meta-analysis was that an increase in G:F due to higher concentrations of Gly_{equi} was lower the higher the ratio between Met and TSAA (Met:TSAA) was. In contrast, an increase in G:F due to higher concentrations of Gly_{equi} was more pronounced the higher the concentration of Cys was. Thus, the meta-analysis confirms the interactive effects found by Powell et al. (2011) and describes these interactions on a quantitative basis. The results of the meta-analysis also confirmed the absence of an interactive

effect between the Gly_{equi} concentration and Met:TSAA ratio or Cys concentration with regard to the ADG found by Powell et al. (2011).

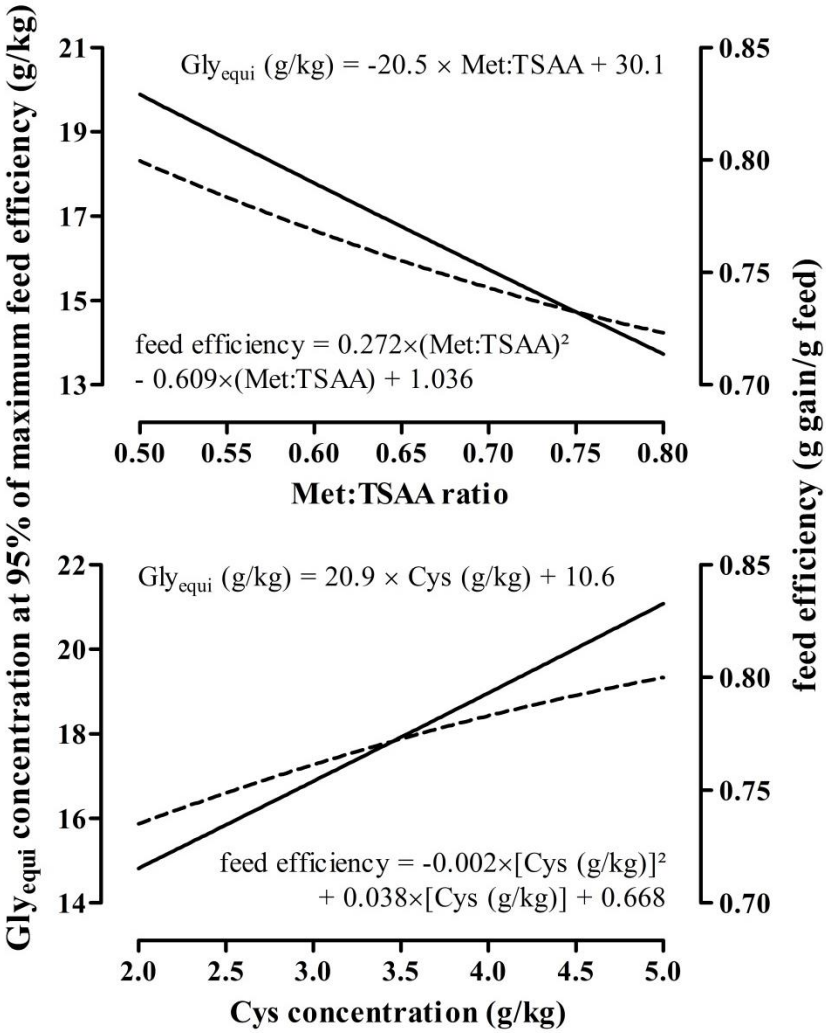

Figure 4-1. Effect of the ratio between methionine and total sulfur amino acids (Met:TSAA) ratio and cysteine (Cys) concentration in diets on feed efficiency at 95% of maximum response (dashed lines) and the required dietary Gly_{equi} concentration at 95% of maximum feed efficiency (solid lines) (based on the data presented in chapter 5.1).

The range of requirement values at 95% of maximum response for dietary Gly_{equi} was similar when the models with the Met:TSAA ratio and the Cys concentration as the second independent variables were used (Figure 4-1 and Figure 4-2). The difference in Gly_{equi} requirement was 1.5 and 1.6 g/kg for ADG and 6.1 and 6.3 g/kg for G:F as response traits for the range of observed Met:TSAA ratios and Cys concentrations, respectively. Thus, these models cover a wide range of dietary Gly_{equi} concentrations at 95% of maximum response; therefore, they prove that the

concentrations of both Met and Cys have a considerable impact on growth response to dietary Gly_{equi}. The comparison of the requirement for Gly_{equi} when the Met and Cys concentrations are not considered (Table 4-2) and the range of Gly_{equi} requirement when the Met:TSAA ratio and Cys concentration were used as second independent variables (Figure 4-1 and Figure 4-2) emphasizes this aspect.

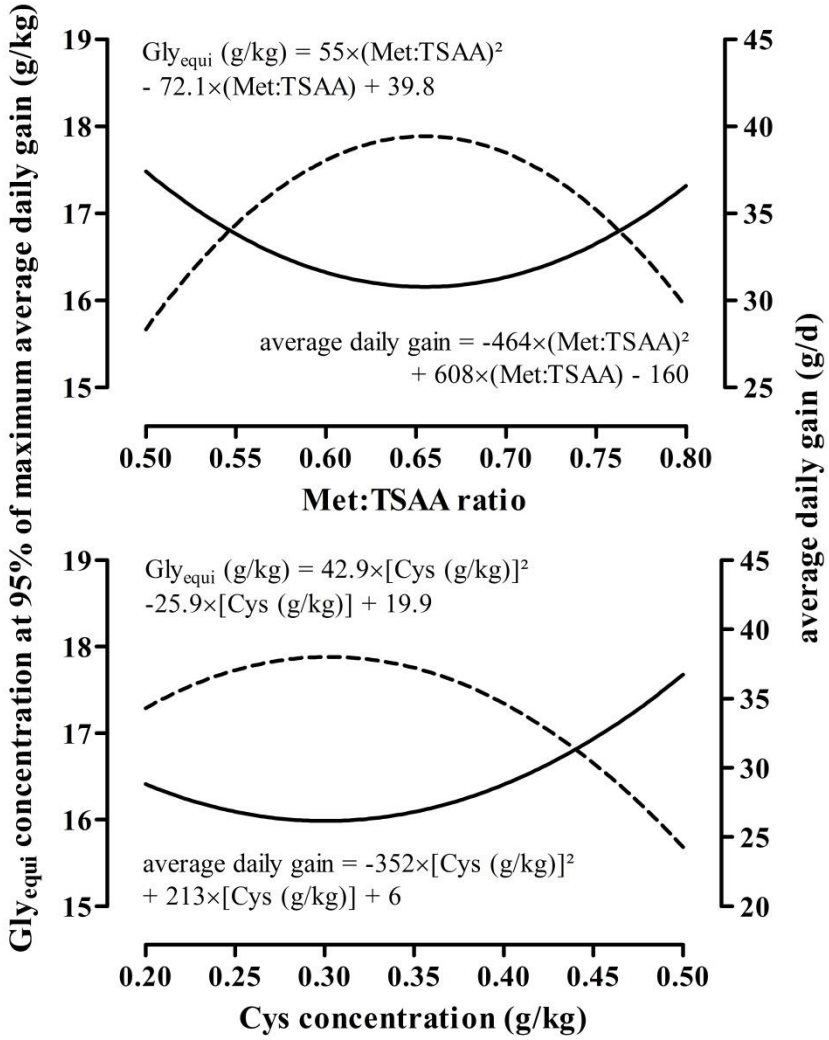

Figure 4-2. Effect of the ratio between methionine and total sulfur amino acids (Met:TSAA) ratio and cysteine (Cys) concentration in diets on average daily gain at 95% of maximum response (dashed lines) and the required Gly_{equi} concentration at 95% of maximum average daily gain (solid lines) (based on the data presented in chapter 5.1).

The requirement for dietary Gly_{equi} at 95% of maximum G:F decreased with increasing Met:TSAA ratios and increased with increasing Cys concentrations in nonlinear relationships. The linear relationship between the Met:TSAA ratio or Cys concentration and the Gly_{equi}

requirement at 95% of maximum G:F might indicate that a necessity to synthesize Cys from Met existed throughout the observed span of Met:TSAA ratios and Cys concentrations. It is also possible that these linear relationships indicate that the differences in Gly_{equi} requirement were particularly a consequence of Cys formation from Met.

The extent of the effect of the supply with Met and Cys on the response to dietary Gly_{equi} in parts can probably be explained by the following consideration: When Ser is utilized to form Cys from Met, the amino group of Ser is incorporated into the L,L-cystathione molecule (see chapter 2.2.2). Therefore, this Ser molecule is lost for alternative utilization in AA metabolism. Furthermore, the conversion of one molecule of L,L-cystathione to one molecule of L-Cys produces a surplus of one molecule of N in the form of ammonia, which has to be detoxified to uric acid. The formation of each molecule of uric acid requires one molecule of Gly to build up the purine ring. Fulfilling the requirement for both Met and Cys in birds reduces the necessity of the conversion of Met to Cys. Each molecule of Met not converted to Cys reduces the requirement for two units of Gly_{equi} and thus two molecules of N.

With ADG as a response trait the interaction between the Met:TSAA ratio or the Cys concentration and the Gly_{equi} concentration was not significant. However, ADG depended independently on both the Gly_{equi} concentration and the Met:TSAA ratio or the Cys concentration. The dietary Gly_{equi} requirement at 95% of maximum ADG varied in the observed range of Met:TSAA ratios and Cys concentrations (Figure 4-2). This variation in Gly_{equi} requirement was not a consequence of different ADG response to dietary Gly_{equi} as a result of different Met:TSAA ratios or Cys concentrations, but rather a consequence of varying ADG in response to different Met:TSAA ratios or Cys concentrations.

A Met:TSAA ratio and Cys concentration that caused the highest ADG response could be determined at 0.655 and 3.02 g/kg, respectively, because of the lack of an interaction with Gly_{equi} concentration. At these values for the Met:TSAA ratio and Cys concentration the requirement for Gly_{equi} was 16.0 and 16.1 g/kg at 95% of maximum ADG, respectively.

A potential effect of the CP concentration on the requirement for Gly has been discussed previously (Heger and Pack, 1996). Using G:F and ADG as response traits, an influence of the CP concentration in feed on the effect of Gly_{equi} concentration was found in the meta-analysis. The results were similar to the effect of the Met:TSAA ratio and the Cys concentration on the response to the Gly_{equi} concentration, but the variability within the measured range was lower. This was probably related to diet formulation because the concentration of Cys usually increases as the concentration of CP increases. Low concentrations are usually associated with the inclusion of pure ingredients, such as DL-Met. As Cys is usually not added, the Met:TSAA

ratio in diets is increased upon supplementing DL-Met as depicted in a model calculation in Table 4-3. This effect was found in the data set used for the meta-analysis by correlation coefficients between the CP concentration, and Met:TSAA and Cys concentration of -0.59 ($P < 0.001$) and 0.58 ($P < 0.001$), respectively. Therefore, the effect of the CP concentration on the response to Gly_{equi} concentration in parts can be explained by a different necessity to synthesize Cys from Met.

Table 4-3. Model calculation for the effect of crude protein concentration on the Met:TSAA ratio and Cys concentration in feed when the sum of the concentrations of Met and Cys is maintained at 8.7 g/kg dry matter by supplementing free DL-Met.[1]

Crude protein in feed (g/kg)	240	220	200	180	160
Feed ingredients (g/kg of feed)					
Corn	453	515	578	641	721
Soybean meal	418	365	309	250	172
Nutrient supply from corn and soybean meal in feed (g/kg)[2]					
Met	3.35	3.13	2.87	2.60	2.24
Cys	3.62	3.37	3.11	2.83	2.45
TSAA	6.97	6.50	5.98	5.43	4.69
Supplemented free DL-Met (g/kg)	1.73	2.20	2.72	3.27	4.01
Met in feed (g/kg)	5.08	5.33	5.59	5.87	6.25
TSAA in feed (g/kg)[3]	8.70	8.70	8.70	8.70	8.70
Met:TSAA ratio	0.584	0.612	0.642	0.675	0.718

[1] Met = methionine, Cys = cysteine, TSAA = total sulfur amino acids
[2] Concentrations of Met and Cys in corn and soybean meal are taken from Evonik (2010)
[3] Calculated to meet the arithmetic mean of the recommendations for day 1 to 21 post-hatch of the GfE (1999)

Endogenous precursors

Several substances can be metabolized to Gly (chapter 2.2.1). A high potential for conversion of Thr and choline to Gly has been reported in humans and rats (Meléndez-Hevia et al., 2009). Other endogenous precursors have no relevance in broiler feeding or the potential quantity of Gly production is low (Meléndez-Hevia et al., 2009). Therefore, this chapter will deal with the extent to which dietary Gly_{equi} can be replaced by dietary Thr and choline without influencing the level of response.

In the literature, the existence of interactive effects between Thr and Gly+Ser in diets has been reported (Table 4-4). Differences between the studies might arise from different age ranges, nutrient concentrations, and nutrient ranges.

The study presented in chapter 5.2 was carried out in order to quantify the relevance of dietary Thr for the response to dietary Gly_{equi}. As a consequence of different ADFI, the intake of the nutrients under study differed among replicates. Since intake better described the supply of nutrients to animals than the dietary concentration the intake of nutrients was used as independent variables to analyze the data. Different levels of G:F and ADG were achieved with distinct combinations of Gly_{equi} and Thr intake. For ADG, this replacement effect was nearly linear throughout the measured range of Gly_{equi} and Thr intake, whereas the replacement value was nonlinear for G:F.

Several methodological differences between the study presented in chapter 5.2 and the studies mentioned in Table 4-4 might explain differences in the results. The previous studies used the concentration of Gly+Ser as a reference unit, whereas in chapter 5.2 Gly_{equi} was used. However, in all mentioned studies, various concentrations of Gly+Ser or Gly_{equi} were achieved only by adding free Gly. Furthermore, the nutrient range (11.9 g/kg DM Gly_{equi} and 4.3 g/kg DM Thr) was considerably wider in chapter 5.2 than in the studies mentioned in Table 4-4, which makes differences more likely to be determined. The most evident difference is that the studies mentioned in Table 4-4 evaluated the nutrient concentrations as independent variables, whereas in chapter 5.2 the actual intake of nutrients was used.

One molecule of Thr can be converted to one molecule of Gly. Given the molar weights of both molecules, the replacement value due to the endogenous conversion of one mass unit of Thr cannot exceed 0.63 mass units of Gly. In chapter 5.2, the calculated replacement value exceeded this theoretical value by a multiple. According to an own recalculation of the data presented by Ospina-Rojas et al. (2013b), which is described in detail in the discussion of chapter 5.2, the replacement value of Thr to Gly+Ser in the study of Ospina-Rojas et al. (2013b) cannot be attributed only to the endogenous conversion of Thr to Gly as well. In chapter 5.2, the level of the replacement value was most likely due to an excess supply of essential AA. The dietary concentrations of essential AA were calculated at 110% of the GfE (1999) recommendations. If Thr limited growth performance, then the excess essential AA had to be catabolized. The ammonia released during AA catabolism had to be converted to uric acid; here, the formation of uric acid requires Gly (chapter 2.2.2). Thus, increasing the Thr intake probably reduced the amount of catabolized AA, except for Thr, and thereby reduced the need for Gly for uric acid formation. In theory, the additional Gly requirement for uric acid synthesis

accounts for 183 mg per additional percentage point of the recommended AA levels of the GfE (1999) if all essential AA, except Thr, were oxidized (As most studies used for the meta-analysis presented in chapter 5.1 gave the concentrations of dietary Thr, Thr was considered as a second independent variable there. However, because most studies defined the level of essential AA to meet or exceed the recommendations of the NRC (1994), variations in the dietary concentration of Thr were low, leading to results that were not reliably estimable.

Table 4-5). An increase in G:F that is not proportionally accompanied by an increase in ADG indicates increased nutrient utilization (Powell et al., 2011). This supports the hypothesis of an influence of the ratio of all essential AA, except Thr, to Gly_{equi} and Thr on the replacement value of Thr for Gly_{equi} because the replacement value of Thr for Gly_{equi} was lower at high Thr intake levels within one level of G:F, but nearly linear for ADG. The hypothesis should be tested in a future experiment evaluating replacement values of dietary Thr to Gly_{equi} at different concentration levels of essential AA other than Thr.

Table 4-4. *P*-values, other statistical parameters, and ranges of nutrient concentrations of studies investigating the effect of dietary Gly+Ser and Thr in two-factorial arrangements.[1]

	Response trait	Ospina-Rojas et al., 2013b		Ospina-Rojas et al., 2013a	Corzo et al., 2009
Age range (days post-hatch)		1 to 7	1 to 21	23 to 35	21 to 42
P values					
Gly+Ser effect	BWG	ns	ns	linear[2]	ns
	G:F	ns	ns	linear	ns
Thr effect	BWG	0.045	0.001	0.08	ns
	G:F	0.001	0.021	0.02	ns
Interaction[2]	BWG	0.010	ns	ns	0.05
	G:F	0.010	ns	ns	0.05
Dietary concentrations (g/kg)					
Gly+Ser range		18.0 to 22.5	18.0 to 22.5	14.4 to 17.6	14.4 to 17.6
Thr range		9.3 to 10.7	9.3 to 10.7	8.4 to 9.2	7.2 to 8.1

[1] Gly = glycine, Ser = serine, Thr = threonine, BWG = body weight gain; G:F = feed efficiency, ns = not significant ($P > 0.05$)
[2] Significant ($P < 0.05$) linear effect of the Gly+Ser concentration
[3] Interaction between Gly+Ser and Thr

As most studies used for the meta-analysis presented in chapter 5.1 gave the concentrations of dietary Thr, Thr was considered as a second independent variable there. However, because

most studies defined the level of essential AA to meet or exceed the recommendations of the NRC (1994), variations in the dietary concentration of Thr were low, leading to results that were not reliably estimable.

Table 4-5. Model calculation for the additional Gly requirement for uric acid synthesis per percentage point of the recommended AA levels of the GfE (1999) if all essential AA, except threonine, were oxidized.[1]

	137	mg N[2]
/	0.776	mass proportion of N in NH_4^+
=	176	mg NH_4^+
/	18.0	mg/mmol NH_4^+
=	9.766	mmol NH_4^+
/	4	molecules of NH_4^+ per molecule of uric acid (Wu, 2009)
=	2.442	mmol uric acid
×	1	molecule of Gly per molecule of uric acid (Wu, 2009)
=	2.442	mmol Gly
×	75.1	mg/mmol Gly
=	183	mg Gly

[1] Gly = glycine, AA = amino acid

[2] Additional concentrations of essential AA, except threonine, per percentage unit of recommended levels are 1220 lysine, 440 methionine, 440 cysteine, 190 tryptophan, 1300 arginine, 840 isoleucine, 1340 leucine, 1170 valine, 400 histidine, 790 phenylalanine, and 640 tyrosine, each expressed in mg/kg dry matter. This results, in addition, to N concentrations of 234 due to lysine, 41 due to methionine, 51 due to cysteine, 26 due to tryptophan, 418 due to arginine, 90 due to isoleucine, 143 due to leucine, 140 due to valine, 108 due to histidine, 67 due to phenylalanine, and 49 due to tyrosine, each expressed in mg/kg dry matter.

Studies evaluating possible interaction effects between Gly and choline are not available in the literature despite the prominent role of Gly in poultry. Thus, the results of the study presented in chapter 5.2 concerning the effect of choline cannot be compared to the literature.

Compared to Gly_{equi} and Thr, the effect of choline was low but still had a considerable influence on the Gly_{equi} and Thr intake required to achieve certain levels of response. At lower levels of G:F and ADG ($\leq$ 0.81 g/g and $\leq$ 48 g/d, respectively), the replacement value of Thr for Gly_{equi} intake appeared to be unaffected by the choline intake level. At higher G:F and ADG levels, the replacement value was not parallel between choline intake levels. These alterations in response reflect three-way interactions among Gly_{equi}, choline, and Thr intake. As observed for Thr and Gly_{equi}, the endogenous metabolism of choline to Gly alone cannot explain the extent of the influence of different choline intake levels on the required Thr and Gly_{equi} intake

to achieve certain levels of response. One mass unit of choline can explain the replacement of up to 0.54 mass units of Gly because one molecule of choline can be converted to one molecule of Gly (Soloway and Stetten Jr., 1953).

Betaine is an intermediate step when choline is endogenously synthesized to Gly (Velíšek and Cejpek, 2006). Choline per se is necessary for the formation of acetylcholine and phosphatidylcholine, but dietary choline can partly be replaced with supplemented betaine without differences in the growth performance of broilers (Dilger et al., 2007). Betaine formation from choline is irreversible. Therefore, choline cannot be synthesized from Gly via reversed reactions but choline can also be formed from Ser by another metabolic pathway in a nine-step reaction (chapter 2.2.1). However, Gly can be converted to betaine with dimethylglycine as an intermediate step via the enzymes glycine-sarcosine methyltransferase (EC 2.1.1.156) and dimethylglycine N-methyltransferase (EC 2.1.1.161) (Meléndez-Hevia et al., 2009). It is possible that one or more of these reactions was rate-limiting in the study presented in chapter 5.2, as found for choline oxidation to betaine in pigs (Siljander-Rasi et al., 2003). This might partly explain the extent of the replacement values of choline to Gly_{equi}. Simultaneous evaluation of different dietary combinations of Gly, choline and betaine, possibly in an experimental design analogous to the study described in chapter 5.2, should reveal further insight into the inter-relationship between those nutrients for broiler nutrition.

As discussed above, the data of the study described in chapter 5.2 were evaluated using the intake of Gly_{equi}, choline, and Thr as independent variables because the intake described the supply of nutrients to animals better than the dietary concentrations. However, determining nutrient intake is impractical and dietary concentrations are required for industry applications. Therefore, regression analyses were conducted to identify the dietary nutrient concentrations that caused certain intake levels. Based on these regression analyses, combinations of Gly_{equi}, choline, and Thr concentrations that caused 95% of maximum G:F and ADG could be determined. At a medium choline concentration of 1.36 g/kg DM, which corresponds approximately to the recommendations of the GfE (1999), Gly_{equi} concentration followed the exponential functions

$$Gly_{equi}\ (g/kg\ DM) = 1{,}580 \times e^{[-0.713 \times Thr\ (g/kg\ DM)]} + 7.05 \text{ for G:F } (R^2 > 0.999) \text{ and} \quad (1)$$

$$Gly_{equi}\ (g/kg\ DM) = 243 \times e^{[-0.290 \times Thr\ (g/kg\ DM)]} - 0.8 \text{ for ADG } (R^2 > 0.999) \quad (2)$$

within the observed range of 6.9 to 7.7 g/kg DM and 7.7 to 9.1 g/kg DM dietary Thr for G:F and ADG, respectively. The replacement values of Gly_{equi} to Thr for different choline concentrations per additional 0.1 g/kg DM Thr ranged from 1.00 to 0.45 g/kg DM Gly_{equi} for

G:F and 0.72 to 0.45 g/kg DM Gly_{equi} for ADG at 95% of maximum response (Figure 4-3). This demonstrates that assuming constants would be simplifying for both response traits.

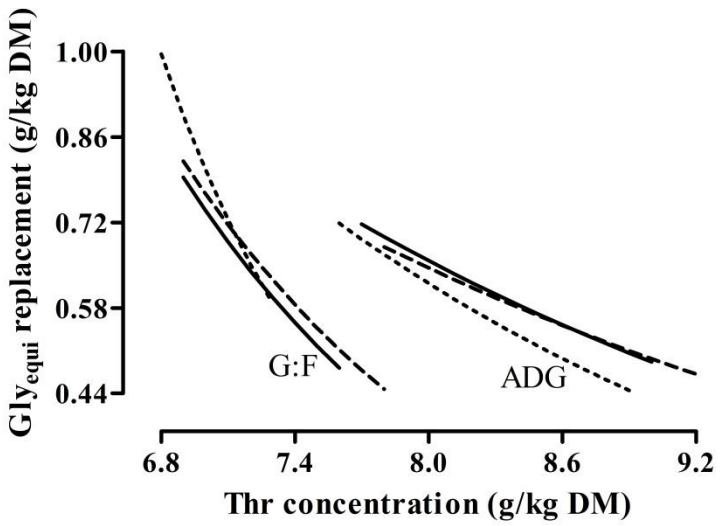

Figure 4-3. Gly_{equi} replacement of 0.1 g/kg dry matter (DM) additional threonine (Thr) at different dietary Thr concentrations at fixed choline concentrations of 1.03 g/kg DM (dashed line), 1.37 g/kg DM (solid line), and 1.72 g/kg DM (dotted line) for feed efficiency (G:F) and average daily gain (ADG) at 95% of maximum response (based on data presented in chapter 5.2).

The magnitude of the replacement effects of Gly_{equi}, choline, and Thr on each other emphasizes the necessity to consider the other nutrients when the requirement of one of those nutrients is derived singly. To give examples based on the data presented in chapter 5.2: At a fixed choline concentration of 1.05 g/kg DM (choline concentration at which maximum G:F was determined), the Thr requirement at 95% of maximum G:F was 8.2, 8.8, and 9.3 g/kg DM for dietary Gly_{equi} concentrations of 19.5, 21.2, and 22.9 g/kg DM, respectively (Figure 4-4a). Likewise, at a fixed Gly_{equi} concentration of 19.5 g/kg DM, the Thr requirement at 95% of maximum G:F was 9.5, 9.3, and 8.8 g/kg DM for dietary choline concentrations of 1.3, 1.37, and 1.72 g/kg DM, respectively (Figure 4-4b). This might partly explain different results of studies investigating the Thr requirement of broilers (e.g. Kidd et al., 2004; Mehri et al., 2014; Taghinejad-Roudbaneh et al., 2013) and the variable response to dietary choline described by the NRC (1994).

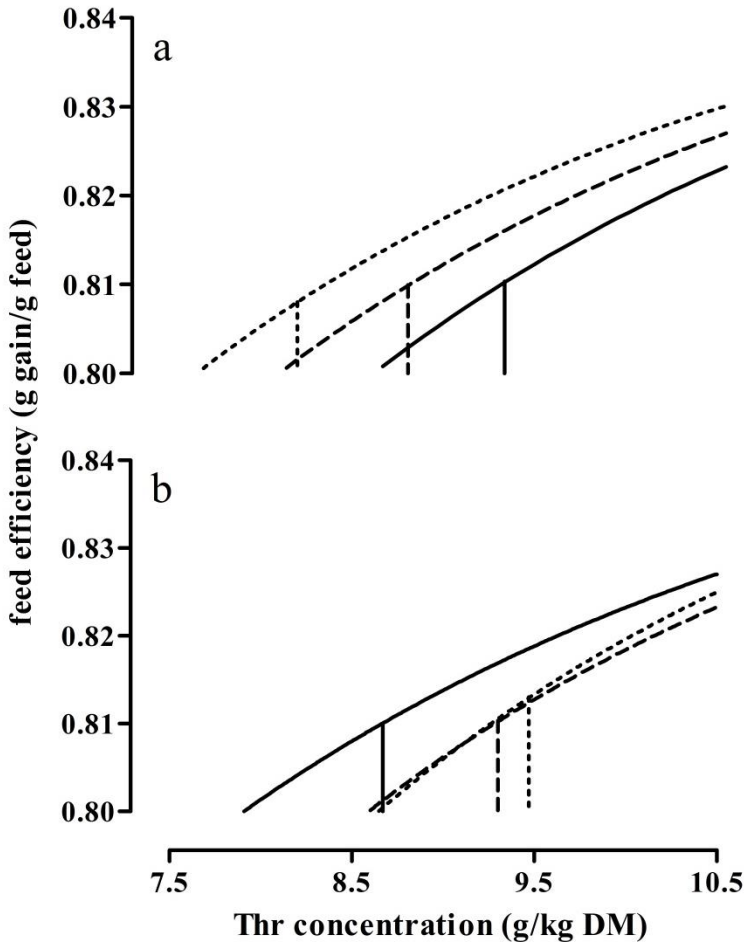

Figure 4-4. Effect of dietary threonine (Thr) concentration on feed efficiency at different levels of dietary Gly_{equi} at a fixed choline concentration of 1.05 g/kg dry matter (DM) (a) and choline at a fixed Gly_{equi} concentration of 19.5 g/kg DM (b) based on the results presented in chapter 5.2. The dietary Thr requirement at 95% of maximum feed efficiency is depicted by the vertical lines.

Proportion of free amino acids in diets

The variation in growth performance responses to dietary Gly_{equi} in the literature may be partly due to between-study differences in animal requirements for uric acid synthesis, as suggested by Namroud et al. (2008). Free AA are absorbed faster into the systemic circulation than peptide-bound AA (Krehbiehl and Matthews, 2003). An imbalance between AA in the systemic circulation as a consequence of faster passage of free AA through the intestinal wall might occur and lead to an increased amount of certain AA catabolized in the enterocytes (see chapter 2.1.4). Ammonia resulting from the catabolism of AA is a toxic agent that has to be detoxified to uric acid in avian species. One molecule of Gly is needed to form the purine ring of one molecule of uric acid when Gly and β-phosphoribosyl-1-amine react to glycinamide

ribotide (Seegmiller, 1975; Sonne et al., 1946). A different growth response to dietary Gly_{equi} in consequence of a different utilization of Gly for uric acid has been surmised in the literature when diets with different proportions of free AA have been fed to broilers (Namroud et al., 2008). However, existing literature is lacking specific investigations concerning this aspect. The study presented in chapter 5.3 was carried out to investigate whether different proportions of peptide-bound and free AA in diets influence broiler Gly_{equi} requirements.

Growth performance was different when a soy protein isolate as an AA source providing peptide-bound AA or a mix of free AA which supplied the same amount of 18 proteinogenic AA was fed. Replacing AA from the soy protein isolate considerably reduced ADFI and, therefore, ADG by 35 and 49%, respectively (see chapter 4.3). Raising the Gly_{equi} concentration increased ADG in both AA sources but the cause was different. High Gly_{equi} concentrations caused ADFI to increase when soy protein isolate as the AA source was fed, whereas G:F was not affected. Conversely, high Gly_{equi} concentrations did not affect ADFI but increased G:F with the AA mix as the AA source. The high Gly_{equi} concentration may have increased ADFI in treatments containing the soy protein isolate because Gly_{equi} was deficient in the low Gly_{equi} concentration and AA deficiency reduces feed intake (Picard et al., 1993). The overall high level of G:F in the treatments with soy protein isolate (approximately 106% of the breeder's objectives (Aviagen, 2014) may explain why the Gly_{equi} concentration had no effect. In the treatments with the AA mix, the Gly_{equi} concentration may not have influenced ADFI because the low Gly_{equi} concentration did not cause the low ADFI. More adequate Gly_{equi} concentrations may have increased the G:F of the treatments with the AA mix partly because of lower endogenous Gly_{equi} synthesis.

Increased ammonia detoxification to uric acid resulting from AA catabolism would be reflected by lower N efficiency, because the Gly required for uric acid synthesis is no longer available for N accretion. In this study, Gly_{equi} concentration had no effect on N efficiency in either AA source. In the treatments with soy protein isolate, the lower ammonia-N/(ammonia-N + uric acid-N) ratio at high Gly_{equi} concentrations showed that the extra Gly_{equi} was used in part for uric acid synthesis. In the treatment with the low Gly_{equi} concentrations, more urinary N probably was excreted as ammonia. In contrast, Gly_{equi} concentration had no effect on the ammonia-N/(ammonia-N + uric acid-N) ratio in the treatments with the AA mix. This result indicates that the extra Gly_{equi} was not used for uric acid synthesis. These observations are not in line with the hypothesis that more Gly is required for uric acid synthesis when high amounts of free AA are used in the feed. This discrepancy might be explained by other processes limiting

the formation of uric acid in the treatments containing the AA mix. Hypotheses for such other processes are described in chapter 4.3.

It remains unclear whether the inclusion of free AA influences the Gly_{equi} requirement at an ADFI equal to the one found in treatments with soy protein isolate. This aspect should be further investigated if it is possible to overcome the reason that caused the reduced ADFI in the treatments with the free AA mix.

4.2.2 Further areas of investigation

Interrelationship of Gly, Arg, guanidino acetic acid, and creatine

Arg and Gly are precursors of guanidino acetic acid, which further reacts to form creatine (see chapter 2.2.2). Creatine, although the active component, is not an ideal feed additive due to its instability and cost, whereas its precursor guanidino acetic acid has more preferable characteristics in terms of stability and price (Baker, 2009). Significant interaction effects for combinations of two components out of creatine, guanidino acetic acid, Arg, and Gly on growth and feed efficiency were reported for broilers in several studies (amongst others Austic and Nesheim, 1972; Dilger et al., 2013; Ringel et al., 2008; Savage and O'Dell, 1960; Waterhouse and Scott, 1960). This indicates that a lower proportion of endogenously metabolized guanidino acetic acid might optimize the metabolic processes in broilers. Simultaneous evaluation of different dietary combinations of Gly, Arg, and guanidino acetic acid, possibly in an experimental design analogous to the study described in chapter 5.2, should reveal further insight into the inter-relationship between those nutrients for broiler nutrition. Thereby, further explanations for different response to dietary concentrations of Gly and Ser might be revealed.

Fat digestibility and energy

Supplementation of Gly was found to increase fat digestibility in broilers (Alzawqari et al., 2010; Ospina-Rojas et al., 2013a) and laying hens (Han and Thacker, 2011). Ospina-Rojas et al. (2013a) also demonstrated that the apparent metabolizable energy concentration (without correction for the N accretion) in diets was increased because the gross energy content in the excreta was reduced as a consequence of higher fat digestibility. This might partly explain the increased abdominal fat deposition observed by Yamazaki et al. (2006) when diets were supplemented with Gly. At present, excessive fat deposition generally is unfavorable because it reduces carcass yield and consumer acceptance (Fouad and El-Senousey, 2014). If the apparent metabolizable energy concentration of feed is elevated due to an increased dietary Gly

concentration, contribution of energy from other feed ingredients may be reduced in order to avoid increasing fat deposition. Causal connections need to be clarified in further studies and the underlying effects should be quantified. A 2×2 factorially arranged experiment investigating the effect of two dietary concentrations of Gly_{equi} at two energy levels on growth performance, fat digestibility, AME_N concentration, and abdominal fat deposition may reveal further knowledge.

Age effects

Coon et al. (1974) determined higher dietary Gly and Ser concentrations for optimal growth and feed conversion from day 1 to 5 than from day 6 to 15 post-hatch. Ngo et al. (1977) showed that the Gly concentration in the feed of young broilers is linked to the creatine concentration of muscle tissue. They found that the increase in the creatine concentration in the pectoral muscle in chicks was pronounced from day 3 to 7 and moderate from day 7 to 14 post-hatch, whereas from then on until day 21, the increase of the concentration was marginal and denoted a plateau. This can be related to age effects or other dietary characteristics influencing the accretion of creatine (chapter 2.2.2). In the study of Schutte et al. (1997) the dietary Gly_{equi} concentration necessary to achieve maximum ADG and G:F was not substantially different between day 1 to 14 and between day 1 to 21 post-hatch (16.8 and 16.7 g/kg for ADG and 1.89 and 1.85 g/kg for G:F). The same animals were used for the evaluation of both time periods. Different responses from day 1 to 21 post-hatch might have been undetectable because of different initial situations as a consequence of different ADG and G:F from day 1 to 14 post-hatch. Clear evidence for an increased requirement for Gly or Ser during the first days of age currently cannot be derived from the literature.

The hypothesis of a higher requirement for Gly_{equi} during the first days of age might originate from the development of G:F in this age period. A model calculation shows that the required Gly_{equi} concentration in feed decreases with advancing age of broilers mainly as a consequence of decreasing G:F (Figure 4-5). Targeted investigations appear reasonable.

4.2.3 General considerations

Waguespack et al. (2009a) and Ospina-Rojas et al. (2014) suggested Gly+Ser as the fourth-limiting and fourth- or fifth-limiting AA in diets based on corn and soybean meal with 17.8 and 16% CP, respectively (chapter 2.1.3). Gly and Ser might be at another rank of limiting AA when the dietary concentrations of Met, Cys, and Thr differed because of a potentially lower or higher requirement for Gly and Ser. The dietary concentration of Arg may also have an

influence. However, this aspect is not assessable because both Waguespack et al. (2009a) and Ospina-Rojas et al. (2014) did not give the concentrations of Met and Cys separately and Thr concentration was not varied.

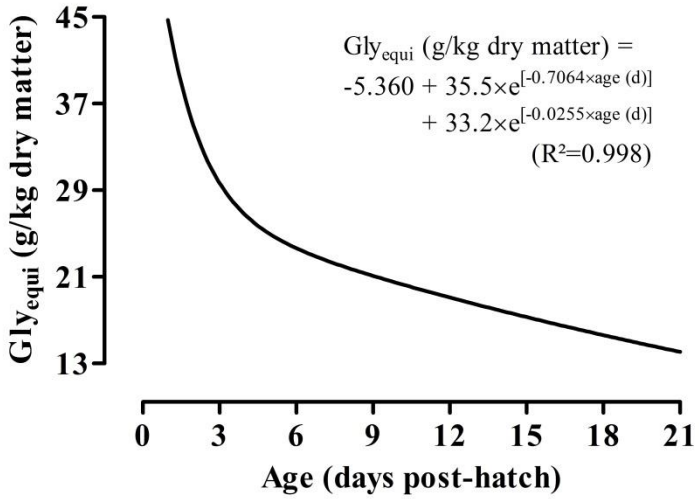

Figure 4-5. Model calculation for the required Gly_{equi} concentration in feed dependent on age based on the growth performance of breeder's objectives (Aviagen, 2014), the glycine and serine accretion described by Fatufe et al. (2004) and Fatufe and Rodehutscord (2005) assuming a utilization of glycine and serine in feed for accretion of 60%, and a dry matter content in feed of 88%.

4.3 Evaluation of the utilization of peptide-bound and free amino acids

Free AA are often incorporated into diets to reduce CP and AA concentrations. At present, feed formulation for broilers assumes equal utilization of free and peptide-bound AA. However, free AA are absorbed faster into the systemic circulation than peptide-bound AA (Krehbiehl and Matthews, 2003). In the literature, an imbalance between AA in the systemic circulation as a consequence of faster passage of free AA through the intestinal wall is often hypothesized to occur. An imbalance between AA would lead to an increased amount of certain AA being catabolized (see chapter 2.1.4), which would be lost for further utilization. This would reduce growth performance and N efficiency. The results of the study presented in chapter 5.3 targeted this hypothesis. Broilers fed with a soy protein isolate had a higher ADG and G:F than broilers fed with an free AA mix that supplied the same amount of 18 proteinogenic AA. N efficiency was not influenced by the AA source on day 21, despite the lower growth rate of the broilers

fed the AA mix. Thus, the reduced ADFI of the broilers fed the free AA mix was determined as the main cause for reduced ADG and G:F. The availability of AA for protein synthesis after ingestion did not appear to limit growth performance or N accretion. Free AA may have passed into the systemic circulation faster than peptide-bound AA. However, this effect only produced minor differences in N efficiency.

Approximately 45% of the CP concentration was provided by either soy protein isolate or the mix of free AA. This high level was selected because the main target of this study was to investigate whether the proportion of free AA in diets has an effect on the response to dietary Gly_{equi}. So far, it is unknown whether ADFI decreases proportionally with the replacement level of peptide-bound to free AA or if ADFI is unaffected to a certain replacement level. Decreasing ADFI has been reported in several studies (e.g. Dean et al., 2006; Namroud et al., 2008, 2010) when an increased proportion of free AA was fed but not in others (e.g. Corzo et al., 2005; Powell et al., 2009). In these studies the supplemented free AA were added to diets to achieve similar concentrations of essential AA to diets with higher CP concentrations. The concentrations of nonessential AA thereby were decreased. Thus, effects on ADFI cannot be attributed singly to the proportion of free AA in diets. Further investigations evaluating different replacement levels of peptide-bound to free AA would contribute to provide knowledge of the effect of free AA in diets on ADFI.

No studies have investigated the substitution of peptide-bound AA by free AA in poultry, with few studies existing for other farm animals. Results of Officer et al. (1997, 1998), who replaced 140 g/kg casein with essential free AA of the same pattern in pig feed, but used unequal concentrations of nonessential AA are discussed in detail in chapter 2.1.4. In brief, Officer et al. (1997) found no differences in N efficiency like in the study presented chapter 5.3, whereas N efficiency of pigs fed free AA in a subsequent experiment (Officer et al. 1998) was significantly lower than in pigs fed the casein diet. It remains unclear what caused differences between these studies. Compared to pigs, potential differences in the utilization of free and peptide-bound AA are probably less pronounced in poultry. In the present study, birds had unrestricted access to feed. Because the crop portions the amount of feed delivered to the posterior digestive tract, peaks of certain AA may have been muted in the systemic circulation. This phenomenon might have diminished the necessity to oxidize AA that could not be utilized by the birds.

Both the soy protein isolate and the AA mix contained other compounds than the 18 considered AA that were not equal between the treatments. In soy protein isolate, 126 of the 147 g N/kg DM was explained by the measured concentrations of the 18 AA considered in the

AA mix. Consequently, broilers receiving the diets containing soy protein isolate were provided with 1.53 g N/kg DM of unknown nitrogenous compounds, such as nonproteinogenic AA, purines, nitrogenous glycosides, betaine and choline. The study presented in chapter 5.2 showed that minor variations in choline concentration can have a noticeable effect on growth performance. However, choline in soy protein isolate was unlikely to influence growth performance because the choline content in soybean products is very low according to the tables of the NRC (1994).

Compared to all other treatments, the (ammonia-N + uric acid-N)/total N ratio in excreta was significantly lower on day 14 in the treatments containing soy protein isolate and on day 21 in the treatment containing soy protein isolate with low Gly_{equi} concentration. This result indicates that these treatments caused more N to be excreted in the faeces, with N from uric acid and ammonia representing 81 to 96% of total N in avian urine (Goldstein and Skadhauge, 2000). Greater N contribution by the faeces to total N excretion may be partly explained by higher inevitable endogenous N loss following higher feed intake, because inevitable endogenous N loss is dependent on DM intake (Adedokun et al., 2011). The different contribution of N excretion by the faeces may also be mainly due to the different availability of AA to the animals. Weanling pigs had an apparent N digestibility of soy protein isolate of 85% (Cervantes-Pahm and Stein, 2010), but free AA were found to be completely digestible (Baker, 2009). Compared to the AA in soy protein isolate, the higher availability of AA from the AA mix in the systemic circulation was probably compensated for by increased N disposal via the urine.

The ammonia-N/total N ratio in urine ranges from 7 to 16% under usual conditions (Goldstein and Skadhauge, 2000; Skadhauge 2010). An increase in deamination processes (e.g. due to starvation) or the necessity to excrete acid causes these values to be exceeded (Hamm and Simon, 1987). This is because increased ammonia excretion represents an adaptive response to excrete acid (Patience, 1990). It was unlikely that AA oxidation was required to generate energy because all treatments had high dietary energy content. However, deamination might have been necessary if AA could not be utilized for protein synthesis. Diets with the AA mix contained 1.54 g/kg DM more HCl than the diets containing soy protein isolate because of the presence of L-Lys·HCl, L-Cys·HCl·H_2O and L-Arg·HCl. Quantitatively, each gram of Lys, Cys and Arg added as L-Lys·HCl, L-Cys·HCl·H_2O and L-Arg·HCl in a diet would contribute 7, 5 and 6 mEq/kg DM of acid, respectively (Patience, 1990). Thus, the calculated acid load due to hydrochlorides in the AA mix was 66 mEq/kg DM; however, we did not evaluate whether this acid load challenged the capacity of the birds to maintain acid-base homeostasis.

A glutamine (Gln) or Asn deficiency may also have caused the low ADFI of the treatments with the AA mix. During acid hydrolysis prior to AA analysis, Asn and Gln lose the amide residue in the side group and form Asp and Glu, respectively (Fontane, 2003). Consequently, the measured concentrations of Asp and Glu in the diets containing soy protein isolate also include Asn and Gln. No literature is available about the proportion of Asn to Asp and the proportion of Gln to Glu in soy products. Therefore, soy protein isolate provided an unknown quantity of Asn and Gln, whereas the AA mix only contained Asp and Glu. Two molecules of Gln are required to construct the purine ring of uric acid when α-phosphoribosyl-1-pyrophosphate is transformed to β-phosphoribosyl-1-amine and when α-N-formylglycinomide ribotide is subsequently metabolized to formylglycinomidine ribotide (Seegmiller, 1975). Gln availability limits the rate of uric acid formation (Karasawa et al., 1973). Thus, the conversion of Gln from Glu may have limited the formation of uric acid in the treatments with the AA mix. This response would have led to a lower rate of ammonia removal by uric acid synthesis, which, in turn, would have increased the concentration of ammonia in the systemic circulation and amount of ammonia being excreted. This phenomenon may explain the low or absent effect of Gly_{equi} concentrations on the ammonia-N/(ammonia-N + uric acid-N) ratio, because Gly is required for uric acid formation after the first step involving Gln (Seegmiller, 1975).

Namroud et al. (2008) found that the concentration of blood plasma ammonia was correlated with the concentration of ammonia in the excreta of broilers. In the study presented in chapter 5.3, blood plasma ammonia was measured; however, no differences among treatments were detected because of a wide variation among the repeated measurements. Reduced feed intake by dogs, humans, and rats occured when the blood plasma ammonia concentration increased (Noda, 1975; Chance et al., 1988; Walker, 2009). Thus, increased ammonia concentrations in the blood plasma of broilers fed the AA mix diet may have caused their feed intake to decline. The metabolism of Asn and Gln is linked to the regulation of acid-base homeostasis (Coon and Balling, 1984). Thus, reduced feed intake might have been induced by limited Gln availability for uric acid synthesis and/or might have been the result of disturbed acid-base homeostasis.

The hypothesis of a disturbed acid-base homeostasis is supported by a different pH of the diets containing soy protein isolate and the free AA mix as AA sources because the pH of the feed with the free AA mix was lower than the feed with soy protein isolate (Table 4-6). Thus, the regulatory functions that maintain the acid-base homeostasis possibly were challenged to a higher extent in the treatments containing the free AA mix than in the treatments containing soy protein isolate. Compared to the diet containing soy protein isolate, the buffering capacity toward acidity and alkalinity of the diets containing the free AA mix was higher (Table 4-6).

This may have led to pH differences in the gastrointestinal tract due to a different influence of the hydrogen chloride and bicarbonate secreted by the proventriculus and the pancreas, respectively. This might have contributed to overstraining the regulatory mechanisms for acid-base homeostasis. However, gastrointestinal pH and indicators for a disturbed acid-base homeostasis like pH, hydrogen carbonate, partial pressure of carbon dioxide, and partial pressure of oxygen in blood (Olanrewaju et al., 2015) were not measured.

Table 4-6. Feed pH and buffering capacity of feed with different amino acid sources used in chapter 5.3.

Amino acid source[1]	Soy protein isolate	Free amino acid mix
Feed pH[2]	5.34	4.55
Buffering capacity towards acidity (mmol HCl/l)[3]		
pH 4	1.3	1.6
pH 3	1.5	2.3
pH 2	2.2	3.5
Buffering capacity towards alkalinity (mmol NaOH/l)[3]		
pH 6	0.7	0.9
pH 7	0.8	1.0

[1] Only the values of the amino acid sources are presented because no difference was observed for the Gly_{equi} levels.
[2] Method: 10 g of feed were filled into a volumetric flask and filled with bidistilled water to 100 ml. After 1 h of continuous stirring pH was measured using a pH electrode. The given values are the arithmetic mean after double determination.
[3] Method: A solution of 0.1 moles of HCl or NaOH per liter of bidistilled water was titrated with continuous stirring until the targeted pH was stably reached. The given values are the arithmetic mean after double determination.

Infusion or oral administration of sodium bicarbonate is used in the treatment of a wide variety of causes for a disturbed acid-base homeostasis in humans (Łoniewski and Wesson, 2013). For poultry, Okumura and Tasaki (1968) showed that increasing the concentration of sodium bicarbonate in diets can enable laying hens to maintain acid-base homeostasis when hydrogen chloride was added to the diet. Therefore, investigating whether addition of sodium bicarbonate to a diet with a high proportion of free AA can overcome a decline in ADFI compared to a diet containing mainly peptide-bound AA might provide further insight if a disturbed acid-base homeostasis caused the low ADFI. Whether a deficient supply with Gln or Asn caused the low ADFI in the treatments with the free AA mix can be evaluated by partially replacing free L-Asp and L-Glu by free Asn and Gln, respectively.

The hypotheses of a deficient Gln and Asn supply, and a disturbed acid-base homeostasis appear to be the most likely reasons for low ADFI in the treatments containing the free AA mix. Another hypothesis to explain low ADFI in the treatments containing the free AA mix is a possible aversion of the diets containing the free AA mix due to flavor-related characteristics. Some of the free AA included had a sweet (L-Ala, Gly, L-Ser, and L-Thr), bitter (L-Arg, L-Ile, L-Lys, L-Asp and L-Phe), umami (L-Glu and L-Asp) or flat-to-bitter (L-Cys, L-Met, L-Trp, L-Pro, L-His, L-Leu, L-Tyr, and L-Val) taste in humans (Kawai et al., 2012). When free AA are supplied as hydrochlorides, the AA additionally taste salty and sour. Peptides have different taste characteristics (Wu, 2013). Consequently, the diets containing the soy protein isolate had different taste properties compared to the diets containing the free AA mix. Roura et al. (2013) described a tendency to avoid bitter and sour feed among broilers in choice experiments. Compared to the diet containing soy protein isolate, the taste perception probably was more sour in the diets containing the free AA mix due to the lower pH (Table 4-6), but the effect of taste perception on the feed intake of broilers is largely unknown (Roura et al., 2013). Therefore, it is possible that the voluntary ADFI was different between the AA sources but the taste perception is difficult to measure in birds.

The taste receptor and signaling effector gene expression in the gastrointestinal tract of broilers indicates that pathways for sweet, bitter, and umami taste perception are involved in the identification system of birds sensing AA (Cheled-Shoval et al., 2015; Roura et al., 2013). Maenz and Engele-Schaan (1996) found that most of the free L-Met provided disappeared in the anterior section of the small intestine. Such information for other free AA is not available in the literature. If other free AA disappear in the anterior section like free L-Met, the lower appearance of AA in the subsequent sections of the small intestine would lead to low perception of the taste receptors for AA in the sections of the gastrointestinal tract posterior to the duodenum. This might lead to the perception of a deficiency of AA in the diet, which usually leads to reduced ADFI (Picard et al., 1993). This hypothesis, however, appears unlikely to be the reason for the low ADFI of the treatments containing the free AA mix because important functions like feed intake are usually regulated by several physiological mechanisms that control each other (Bungo et al., 2011).

4.4 Recommended concentrations for glycine and serine in feed

4.4.1 Evaluation of the current situation

Like any nonessential AA, Gly and Ser currently are not considered in the recommendations of the GfE (1999). The NRC (1994) recommends 12.5 g/kg dietary Gly+Ser. This value is a rough estimate after evaluation of five studies published from the 1950s to the 1970s. A wide variation in response to dietary Gly+Ser was pointed out by the authors. Those five studies were conducted with slower-growing chicks than are currently used commercially and with purified diets resulting in requirement estimates from 3 to 18 g/kg (Dean et al., 2006). The lowest Gly_{equi} concentration at 95% of maximum response that could be determined in the meta-analysis presented in chapter 5.1 is above the recommendations of the NRC (1994) of 12.5 g/kg Gly+Ser. Thus, the recommendations of the NRC (1994) appear outdated.

The current Brazilian recommendations give 21.7 g/kg dietary Gly+Ser from day 1 to 7 post-hatch and 19.4 g/kg dietary Gly+Ser from day 8 to 21 post-hatch for male broilers (Rostagno et al., 2011). These recommendations do not state the basis for determination of the values. The recommended concentrations for Gly+Ser are high compared to most of the determined concentrations at 95% of maximum ADG and G:F in the literature (Table 4-2). This might be attributed to an intention of the authors to consider some safety margin for different conditions of factors influencing the response to dietary Gly and Ser.

As is currently common practice, both the NRC (1994) and Rostagno et al. (2011) take the analogue effect of dietary Gly and Ser as the sum of both AA into account, neglecting that Ser only has the same effect as Gly on an equimolar basis. Therefore, according to current knowledge, consideration of Gly_{equi} as the Gly equivalent of those AA appears to describe the effect of a diet more properly than their pure sum. Expressed in Gly_{equi}, the current recommendations of the NRC (1994) offer an interpretation between 8.9 and 12.5 g/kg Gly_{equi} depending on the contribution of Gly and Ser to Gly+Ser. In this respect, the recommendations of Rostagno et al. (2011) cover a range between 15.5 and 21.7 g/kg Gly_{equi} from day 1 to day 7. Considering the ratio between Gly and Ser in commonly used vegetable feedstuff (Table 2-1), a concentration of 12.5 g/kg Gly+Ser covers a range between 10.7 and 11.3 g/kg Gly_{equi} and a concentration of 21.7 g/kg Gly+Ser covers a range between 18.3 and 19.5 g/kg Gly_{equi}. However, when animal-based protein feedstuffs are used this range can be wider (Table 4-1).

Akinde (2014) proposed using a reference unit calculated identical to Gly_{equi} for further recommendations but named it "total dietary glycine activity ($glycine_{TDA}$)". As presented in chapter 2.2.1, Gly can be metabolized from substances other than Ser. There is evidence that at

least Thr and choline in part can also meet the functions of Gly (chapter 5.2). Consequently, only considering Ser as an endogenous precursor of Gly in metabolism makes the term “total” appear inappropriate. Furthermore, the term “glycine activity” implies that only Gly is of physiological relevance, neglecting the role of Ser in animals (chapter 2.2.2).

4.4.2 Perspectives

As Gly and Ser have the potential to limit growth and feed efficiency of broilers, those AA should appear in recommendations suitable for low CP diets because low CP diets might become more important. As described above, use Gly_{equi} appears more applicative than the currently common Gly+Ser because the advantage of rendering the physiological value of diets more precisely should justify the additional calculation effort.

As long as recommendations are simplified in tables, consideration of the various factors influencing the optimal dietary concentrations of Gly and Ser is impractical. Therefore, tabular recommendations need to be universally appropriate for different conditions of influencing factors. If, henceforth, the tabular recommendation system is expanded with flexible components, recommended Gly and Ser concentrations may be given as variables due to underlying dietary characteristics. Variable recommendations can exemplarily be adaptations to the Met:TSAA ratio in combination with the dietary Cys concentration or adaptations to the dietary concentration of endogenous precursors.

Linear adaptation factors have the advantage that adjustments of target nutrient concentrations in feed are easy to calculate because they are constant. The study presented in chapter 5.1 shows that the effect of alteration of the Met:TSAA ratio and Cys concentration on the requirement for Gly_{equi} is not a constant. Likewise, the study presented in chapter 5.2 shows that the replacement values of Thr and choline to dietary Gly_{equi} are also not constants. Therefore, some safety margin would have to be considered when correction factors are defined in order to ensure suitability for universal conditions. This would constrain the possibility to recommend nutrient concentrations as close as possible to the requirement of the animals. A possibility to overcome this constraint is by implementing variable adaptations to achieve recommendations that virtually meet the requirement by nutritional modeling. Sound knowledge about the effect of different concentrations of nutrients on each other is necessary for the generation of such models.

In addition to Thr and choline as endogenous precursors and the Met:TSAA ratio in combination with the Cys concentration other dietary characteristics are conceivable as dietary characteristics that affect the recommended concentrations of dietary Gly_{equi}. Such

characteristics might be the proportion of free AA in feed or dietary characteristics describing the interrelationship between creatine, guanidino acetic acid, and Arg.

4.5 Limits, current status and perspectives of crude protein reduction

4.5.1 Limits

The lower limit of CP concentration in diets is reached when all AA and other nitrogenous nutrients are fed in the concentrations that the animal requires along with high digestibility of those nutrients (Officer et al., 1997). Influences on this lower limit of CP concentration, like period of growth, gender, criterion of response or health status are numerous (Baker, 2009). The complexity of the many interacting factors suggests that future research can further approach but probably not reach the ideal AA composition of diets by diminishing safety margins.

Current research mostly aims to find ways to reduce the CP concentration without compromising the growth and feed efficiency achieved nowadays with common CP concentrations (amongst others Corzo et al., 2005; Dean et al., 2006), which is at about 21 to 22% in diets. If ADG, ADFI and G:F are unaltered, a reduction of CP intake and, thus, N intake increases N efficiency and decreases N excretion on condition that all nitrogenous nutrients are still provided adequately. Inevitable metabolic losses such as AA degradation and partial digestibility of nitrogenous nutrients limit the potential to fully complete N efficiency to 100% (Baker, 2009). Further optimization of nitrogenous nutrients in diets can increase N efficiency and, therefore, determine the lowest possible CP level in diets without adverse effects on growth performance and product quality.

4.5.2 Current status and perspectives

As the CP concentration is not specified on a DM basis in most studies, the following specifications refer to the CP concentrations in diets as fed. Dean et al. (2006) summarized that, in the literature, growth and feed efficiency was reduced in 1 to 21 day-old broilers fed with diets containing less than 19 to 20% CP even when the requirement for essential AA was met. If the concentrations of Gly and Ser were adequate, the growth and feed efficiency of broilers fed diets containing 17 to 18% CP were at the level of diets containing more than 20% CP (Corzo et al., 2004, Dean et al., 2006; Heger and Pack, 1996).

The Met:TSAA ratio is not given in the study of Corzo et al. (2004). As the Met:TSAA ratio was high in the studies of Dean et al. (2006) and Heger and Pack (1996) (0.741 and 0.733 to 0.809, respectively), the requirement for Gly_{equi} probably was increased because Ser is needed for the formation of Cys from Met (chapter 5.1). Other nutrient concentrations influencing the response to Gly_{equi} probably were also suboptimal. Consequently, further reduction of the CP concentration in diets without negative effects on performance should be possible through optimization of both the dietary Gly_{equi} concentration and the characteristics influencing the response to Gly_{equi}. According to a personal estimate, this would enable reducing the CP concentration in diets to within 14 to 15% in diets with 88% DM. However, this is only an estimate and needs further knowledge about characteristics influencing the response to Gly_{equi}. Additional knowledge about the effect of endogenous precursors, a potential influence of the proportion of free AA in diets, the aspects discussed in chapter 4.2.2, and verification investigations are necessary to reliably achieve equal growth performance in such low CP concentrations compared to currently common CP concentrations.

Gly and Ser are the first nonessential AA of which experimentally verified requirement values were quantified. Experimentally verified requirement values for other nonessential AA are not available in the literature. The possibility to further reduce the CP concentration in diets without adverse effects on performance can be expected when the role of other nonessential AA is better understood, and experimentally verified requirement values are known and assessable. This would contribute toward further reducing N excretion and, therefore, diminishing the negative effects of broiler meat production on the environment.

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5 Included Studies

5.1 Study I

Meta-analysis of influence of dietary glycine and serine, with consideration of methionine and cysteine, on growth and feed conversion of broilers

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Abstract

The existing literature is inconsistent with respect to optimal dietary concentrations of glycine (Gly) and serine (Ser) in broiler feed. Therefore, we conducted a meta-analysis to investigate the response of broilers to dietary levels of Gly using a full quadratic model based on mixed model methodology. Response was measured as average daily gain (ADG) (g/d), average daily feed intake (ADFI) (g/d), and feed efficiency (G:F) (g/g). Additionally, the influence of other dietary constituents was evaluated. This meta-analysis was based on a data set comprising a total of 9,626 broilers in 10 peer-reviewed papers that investigated the response of broilers to different dietary concentrations of Gly, achieved by addition of free Gly. The fitted quadratic model, with either Gly+Ser or the calculated glycine equivalent (Gly_{equi}) of both amino acids as the independent variable, revealed that all model terms were significant ($P \leq 0.05$), and hence proved a curvilinear relationship between these independent variables and response traits. The R^2 value and root mean square error confirmed a strong relationship between observed and predicted traits. A comparison of the influence of Gly+Ser and Gly_{equi} on response traits revealed that both approaches produced similar results. Because Gly_{equi} should meet the physiological values of a diet better than Gly+Ser, models with two independent variables were conducted using Gly_{equi}. The second independent variables were methionine (Met):TSAA ratio and the concentrations of cysteine (Cys) and crude protein. In models with one or two independent variables, the impact of dietary Gly on ADFI was low. By contrast, G:F was markedly influenced by dietary Gly; this effect intensified at lower Met:TSAA ratios and higher Cys and crude protein levels. ADG was also a function of Gly_{equi} and the second independent variables. For ADG, an optimal Met:TSAA ratio of 0.655 and Cys concentration of 0.302% was calculated. Following the nonlinear nature of relationship, generally applicable replacement values could not be calculated. However, it was concluded that consideration of dietary Cys can diminish the requirement for Gly_{equi}, and therefore, enable a reduction in the crude protein of broiler diets without limiting growth performance.

5.2 Study II

A quantitative study of the interactive effects of glycine and serine with threonine and choline on growth performance in broilers

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Abstract

Differences in the optimal dietary concentrations of glycine (Gly) and serine (Ser) in broiler diets may be due to levels of endogenous Gly precursors that differ in literature. Therefore, we measured the extent of the interactive effects between equivalents of Gly and Ser (Gly_{equi}) and the endogenous Gly precursors choline and threonine (Thr) on growth performance. A fractional central composite design included concentrations of 15 to 25 g/kg dry matter, 0.6 to 2.0 g/kg dry matter, and 6.4 to 10.4 g/kg dry matter for Gly_{equi}, choline, and Thr, respectively, each in 5 levels. The various concentrations were achieved by adding Gly, choline chloride, and L-Thr to a basal mix. Except for 20 replicates of the central diet, all treatments were tested with 5 replicates, each with 10 birds. Food was provided for *ad libitum* consumption throughout the experiment. The data were evaluated using artificial neural networks. Digestibility was studied for selected diets using separate birds. Since average daily feed intake (ADFI) varied between replicates, the intake of prececal digestible Gly_{equi}, choline, and prececal digestible Thr were more adequate independent variables than the dietary concentration of each amino acid. From day 1 to 7, no treatment effects on G:F and average daily gain (ADG) were detected; subsequent results refer to the period from day 7 to 21. Increasing prececal digestible Thr intake considerably decreased the need for prececal digestible Gly_{equi} to achieve certain levels of feed efficiency (G:F) and ADG. The extent of this effect cannot be explained only by the endogenous metabolism of Thr to Gly. Since essential amino acids were present above the recommended levels, Thr probably limited performance, and excessive intake of other essential amino acids prompted a Gly-dissipating process. Choline exerted a considerable effect on the required intake of prececal digestible Gly_{equi} and prececal digestible Thr to achieve certain levels of G:F and ADG. The results of this study partly explain the previously reported variations in response to dietary Thr, Gly, Ser, and choline.

5.3 Study III

Effect of glycine supplementation in low protein diets with amino acids from soy protein isolate or free amino acids on broiler growth and nitrogen utilisation

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Abstract

1. We investigated whether substitution of amino acids (AA) from soy protein isolate with free amino acids in low crude protein diets influences the growth performance and N utilization in broilers and whether interactions with dietary glycine equivalent (Gly_{equi}) concentration exists.

2. Birds were distributed in two 2×2 factorial arrangements of 48 floor pens containing 10 birds each, plus 48 metabolism cages containing 2 birds each. Experimental feed was provided for *ad libitum* consumption from day 7 to 22. Diets contained either a soy protein isolate at 79 g/kg or a mix of free amino acids, which supplied the same amount of 18 proteinogenic amino acids. A mix of free glycine and L-serine was used to obtain low and high (12.0 and 20.5 g/kg dry matter) levels of dietary Gly_{equi}.

3. Substitution of soy protein isolate with free amino acids reduced the average daily gain and feed efficiency, mainly due to reduced feed intake. Efficiency of N accretion was not influenced by the amino acid source or Gly_{equi} concentration on day 21, possibly due to the lower amino acid digestibility of soy protein isolate and higher urinary excretion of nitrogenous substances in the treatments with the amino acid mix.

4. The average daily weight gain of the treatments with high Gly_{equi} concentration was higher for both amino acid sources. This increase was due to higher average daily feed intake by broilers in the treatments with soy protein isolate and due to the increased feed efficiency in the treatments with the amino acid mix. Broilers exhibited different growth responses to dietary Gly_{equi} between the amino acid sources. However, these responses could not be attributed to the different utilization of Gly_{equi} for uric acid synthesis.

6 Summary

The increasing demand for meat and other animal products along with the global limitation of arable land for crop production is expected to result in a shortage of protein-rich feedstuff. This can have an impact on protein and food prices and affect affordability of food in developing countries. Furthermore, the excretion of nitrogenous compounds has negative effects on the environment because of the risk of nitrogen (N) leakage into the groundwater. Ammonia emissions from livestock enterprises have been associated with a number of environmentally damaging effects. The biggest determinant of ammonia emissions associated with livestock farming is excretion of N. Excretion of N in part is inevitable. However, N excretion can be reduced by avoiding excessive intake of feed protein. At present, there is a substantial lack of knowledge about the requirement of nitrogenous nutrients except for essential amino acids (AA) and the influence of these nutrients on animal physiology. As reported in the literature, this has often led to undesirable effects of low crude protein (CP) diets on growth performance.

This thesis focused on factors influencing the response to the nonessential AA glycine (Gly) and serine (Ser) in low CP broiler feed. A deficiency of those AA has been shown to be one potential reason for reduced growth performance when feeding low CP diets. However, growth response to dietary Gly and Ser in low CP diets was inconsistent in the literature.

In the first study, a meta-analysis was conducted to investigate the response of broilers to dietary levels of Gly equivalents (Gly_{equi}) of Gly and Ser in existing literature using a full quadratic model based on mixed model methodology. In addition, the influence of other dietary nutrient concentrations was evaluated. The meta-analysis was based on a data set comprising a total of 9626 broilers in ten peer-reviewed papers that investigated the growth response of broilers to different dietary concentrations of Gly_{equi}, achieved by addition of free Gly. A curvilinear relationship between Gly_{equi} and the response traits average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F) was found. Model fit characteristics confirmed a strong relationship between observed and predicted traits. The impact of dietary Gly_{equi} on ADFI was low, but response in G:F and ADG varied markedly at different levels of Gly_{equi}. According to the results of this meta-analysis, the currently recommended concentration of the National Research Council (1994) of 12.5 g/kg in diets for broilers during the first three weeks of age appears too low for growth rates that are targeted in current broiler production. The effect of dietary Gly_{equi} depended on the supply of both methionine (Met) and cysteine

(Cys). The G:F and ADG response to dietary Gly_{equi} was higher at lower Met:(Met+Cys) ratios and higher Cys concentrations. This was explained by a decreased necessity of conversion of Met to Cys, for which Ser is required. Adequate concentrations of both Met and Cys in broilers diets probably reduced the necessity of the conversion of Met to Cys. The Gly_{equi} requirement increased with increasing Cys concentrations, but this probably is attributable to an increased Gly_{equi} requirement for protein accretion in consequence of increased growth performance.

The second study was conducted to investigate the influence of threonine (Thr) and choline, which are endogenous precursors of Gly, on the response to dietary Gly_{equi}. A fractional central composite design was used, investigating the effect of different concentrations of Gly_{equi}, Thr, and choline on growth performance of broilers from 7 to 21 days post-hatch. Each treatment was tested in 5 pens of 10 birds each, except for the central diet, which was tested in 20 pens of 10 birds each. An increase in Thr concentration reduced the Gly_{equi} concentration required to achieve certain response levels of G:F and ADG. Choline also exerted a considerable effect on the required Gly_{equi} concentration to achieve certain response levels, but the Gly_{equi} replacing effect of choline was less pronounced than Thr. The observed replacement values of dietary Thr and choline for dietary Gly_{equi} exceeded the possible replacement values calculated by considering endogenous conversion. The high replacement values originated most likely from an excess supply of other essential AA than Thr. If Thr and, to a lower extent, choline limited growth performance, then excessive intake of other AA had to be catabolized, resulting in an increased need for Gly_{equi} for uric acid formation. It was concluded that further studies should take Gly_{equi}, choline, and Thr together into consideration when determining the requirements for these nutrients.

The third study aimed to investigate whether the growth performance and N utilization of broilers are influenced by different proportions of free and peptide-bound AA in diets. This study further was aimed to examine whether the different proportion of free and peptide-bound AA influences broiler Gly_{equi} requirements. The hypothesis was that an increased oxidation of free AA leads to an increased ammonia production, which must be detoxified to uric acid in a Gly-dissipating process. A total of 576 broilers were distributed in two 2×2 factorial arrangements of 48 floor pens containing 10 birds each, plus 48 metabolism cages containing two birds each. Each treatment was tested with 12 replicates in both arrangements. Diets contained either a soy protein isolate at 79 g/kg or a mix of free AA, which supplied the same amount of 18 proteinogenic AA. A mix of free Gly and L-Ser was used to obtain low and high (12.0 and 20.5 g/kg dry matter) levels of dietary Gly_{equi}. The response in growth performance was similar between the birds in metabolism cages and the birds in floor pens. Replacing a

substantial amount of AA from the soy protein isolate with free AA reduced ADG and G:F, mainly due to reduced ADFI. Reasons for the reduced ADFI cannot be identified clearly from the results of this study. The efficiency of N accretion after 14 days of feeding the experimental diets was not different between the AA sources, possibly due to the lower AA digestibility of the soy protein isolate and higher urinary excretion of nitrogenous substances in the treatments with the AA mix. Thus, availability of AA for protein synthesis after ingestion probably did not limit broiler growth rates. The ADG of the treatments with the high Gly_{equi} concentration was higher for both AA sources. This increase was due to higher ADFI by broilers in the treatments with soy protein isolate and due to the increased G:F in the treatments with the AA mix. Contrary to the hypothesis, these responses did not give an indication of different utilization of Gly_{equi} for uric acid synthesis.

In conclusion, the response of broiler chicken to dietary Gly_{equi} depends on other dietary characteristics, like the concentrations of Cys, Thr, choline, and the proportion of free AA in diets. The information described in this thesis contribute to enable further optimization of the dietary Gly_{equi} concentration as well as the other dietary characteristics influencing the response to Gly_{equi}. This enables reducing the CP concentration in diets without adverse effects on growth performance and, therefore, diminishing the negative effects of broiler production on the environment.

7 Zusammenfassung

Der steigende Bedarf an Fleisch und anderen Produkten tierischen Ursprungs bei gleichzeitig global begrenztem landwirtschaftlich nutzbarem Land für die Pflanzenproduktion bewirkt, dass eine Knappheit an proteinreichen Futtermitteln zu erwarten ist. Das kann Auswirkungen auf Preise für Lebensmittel und Proteinfuttermittel haben und vor allem in Entwicklungsländern die Bezahlbarkeit von Lebensmitteln beeinträchtigen. Zudem hat die Ausscheidung von stickstoffhaltigen Substanzen wegen des Risikos von Stickstoffeintrag in das Grundwasser negative Auswirkungen auf die Umwelt. Ammoniakemissionen von tierhaltenden Betrieben werden mit einer Vielzahl von umweltschädigenden Auswirkungen in Verbindung gebracht. Der bedeutendste Einflussfaktor auf nutztierhaltungsbedingte Ammoniakemissionen ist die Ausscheidung von Stickstoff (N). Diese sind zum Teil unvermeidlich. Allerdings kann die Ausscheidung von N durch das Vermeiden von überschüssiger Aufnahme von Protein im Futter vermindert werden. Derzeit besteht eine erhebliche Unkenntnis hinsichtlich des Bedarfs an N-haltigen Nährstoffen abgesehen von essentiellen Aminosäuren (AS) und dem Einfluss dieser Nährstoffe auf die Tierphysiologie. Dies hat in vielen Fällen zu nicht wünschenswerten Auswirkungen rohprotein-(XP)-reduzierter Futtermischungen auf die Wachstumsleistung geführt.

In der vorliegenden Arbeit lag der Fokus auf Einflussfaktoren auf die Auswirkungen der nichtessentiellen AS Glycin (Gly) und Serin (Ser) im Futter für Masthähnchen. Ein Mangel dieser AS wurde als eine mögliche Ursache für eine verringerte Wachstumsleistung beim Verfüttern von Niedrigproteinfuttermischungen gefunden. Allerdings waren die Auswirkungen von Gly und Ser in Niedrigproteinfuttermischungen auf die Wachstumsleistung in verschiedenen Studien unterschiedlich.

In der ersten Studie wurde eine Metaanalyse durchgeführt um die Auswirkungen der Konzentration an Gly-Äquivalenten (Gly_{equi}) von Gly und Ser auf die Wachstumsleistung von Masthühnern in bereits veröffentlichter Literatur mithilfe von gemischten Modellen zu untersuchen. Die Metaanalyse basierte auf einen Datensatz, in dem die Wachstumsleistung von 9262 Masthühnern in zehn begutachteten Veröffentlichungen zusammengefasst war. In diesen Veröffentlichungen wurden Auswirkungen verschiedener Gly_{equi}-Konzentrationen im Futter durch Zugabe von freiem Gly auf die Wachstumsleistung untersucht. Zwischen der Gly_{equi}-Konzentration im Futter und den Zielmerkmalen tägliche Zunahmen (TZ), tägliche Futteraufnahme (TFA) und Futtereffizienz (FE) wurde ein kurvilinearer Zusammenhang

festgestellt. Verschiedene Kennzahlen der Modellgüte bestätigten einen engen Zusammenhang zwischen beobachteten und geschätzten Merkmalen. Die Auswirkung der Gly_{equi}-Konzentration auf die TFA war gering; dagegen waren FE und TZ deutlich beeinflusst. Entsprechend den Ergebnissen dieser Metaanalyse erscheint die derzeitige empfohlene Konzentration an Gly+Ser des National Research Council (1994) von 12,5 g/kg im Futter für die in der Masthähnchenproduktion angestrebte Wachstumsleistung während der ersten drei Lebenswochen zu gering. Die Auswirkung der Gly_{equi}-Konzentration hing von der Versorgung mit Methionin (Met) und Cystein (Cys) ab. Die Auswirkung der Gly_{equi}-Konzentration auf das Niveau der TZ und FE war höher bei geringen Verhältnissen von Met zu Met+Cys und höheren Cys-Konzentrationen. Dies kann durch eine verringerte Notwendigkeit der Synthese von Cys aus Met erklärt werden, für die Serin benötigt wird. Ausreichende Konzentrationen von Met und Cys verringern wahrscheinlich die Notwendigkeit der Umwandlung von Met zu Cys. Dennoch stieg der Gly_{equi}-Bedarf mit höheren Cys-Konzentrationen. Dies ist vermutlich durch einen höheren Gly_{equi}-Bedarf für den Proteinansatz infolge einer höheren Wachstumsleistung bedingt.

Die zweite Studie wurde durchgeführt, um den Einfluss von Threonin (Thr) und Cholin, welche endogene Vorstufen von Gly sind, auf die Auswirkungen von Gly_{equi} zu untersuchen. Mit einem fraktionellen Central Composite Design wurden Auswirkungen verschiedener Konzentrationen an Gly_{equi}, Thr und Cholin auf die Wachstumsleistung von Masthähnchen im Alter von 7 bis 21 Tagen geprüft. Alle Behandlungen mit Ausnahme der Zentralbehandlung des Versuchsdesigns, die mit 20 Abteilen mit jeweils 10 Tieren getestet wurde, wurden mit 5 Abteilen mit jeweils 10 Tieren getestet. Eine Erhöhung der Thr-Konzentration verringerte die benötigte Gly_{equi}-Konzentration um bestimmte Niveaus an TZ und FE zu erreichen. Die Cholin-Konzentration hatte ebenfalls einen erblichen Einfluss auf die Gly_{equi}-Konzentration, die benötigt wurde um bestimmte Niveaus an TZ und FE zu erreichen. Allerdings war der Austauschwert von Cholin zu Gly_{equi} geringer als der von Thr zu Gly_{equi}. Die festgestellten Austauschwerte von Thr und Cholin für Gly_{equi} überstiegen die möglichen Austauschwerte, die durch die endogene Gly-Synthese erklärt werden kann. Die hohen Austauschwerte sind wahrscheinlich durch eine übermäßige Versorgung mit anderen essentiellen AS außer Thr bedingt. Wenn Thr und in geringerem Ausmaß Cholin die Wachstumsleistung begrenzte, mussten andere übermäßig aufgenommene AS katabolisiert werden. Dies führt zu einem höheren Gly_{equi}-Bedarf für die Bildung von Harnsäure. Es wurde gefolgert, dass künftige Studien Gly_{equi}, Thr und Cholin zusammen berücksichtigen sollten, wenn Bedarfswerte für diese Nährstoffe ermittelt werden.

Die dritte Studie wurde durchgeführt um zu prüfen, ob die Wachstumsleistung und N-Verwertung von Masthähnchen vom Verhältnis von freien und peptidgebundenen AS im Futter abhängt. Sie sollte zusätzlich prüfen, ob ein unterschiedlicher Anteil von freien und peptidgebundenen AS im Futter den Gly_{equi}-Bedarf beeinflusst. Die Hypothese war, dass eine erhöhte Oxidation von freien AS zu einer erhöhten Produktion von Ammoniak führt, das bei einem Gly-verbrauchenden Prozess zu Harnsäure entgiftet wird. 576 Masthähnchen wurden in zwei 2×2-faktoriellen Versuchsanordnungen mit 48 Bodenhaltungsabteilen mit jeweils zehn Tieren und zusätzlich 48 Stoffwechselkäfigen mit jeweils zwei Tieren verteilt. In beiden Versuchsanordnungen wurde jede Behandlung mit 12 Wiederholungen getestet. Die Futtermischungen enthielten entweder 79 g/kg eines Sojaproteinisolats oder eine freie AS-Mischung, die die gleiche Menge an 18 proteinogenen AS bereitstellte. Durch eine Mischung aus freiem Gly und L-Ser wurden geringe und hohe (12.0 und 20.5 g/kg Trockensubstanz) Gly_{equi}-Niveaus im Futter erreicht. Die Ergebnisse der Wachstumsleistung waren bei beiden Versuchsanordnungen ähnlich. Der Austausch der AS-Menge des Sojaproteinisolats gegen freie AS verringerte die TZ und FE vor allem durch eine verringerte TFA. Ursachen für die verringerte TFA können nicht eindeutig von den Ergebnissen dieser Studie abgeleitet werden. Hinsichtlich der N-Effizienz gab es keinen Unterschied zwischen den AS-Quellen nach 14-tägiger Verfütterung des Versuchsfutters. Dies lag möglicherweise an einer geringeren AS-Verdaulichkeit beim Sojaproteinisolat und einer höheren Ausscheidung von N-haltigen Substanzen über den Urin bei den Behandlungen mit der freien AS-Mischung. Folglich begrenzte die Verfügbarkeit von AS für die Proteinsynthese nach der Aufnahme vermutlich die Wachstumsleistung nicht. Die TZ bei den Behandlungen mit hoher Gly_{equi}-Konzentration war bei beiden AS-Quellen erhöht. Diese Steigerung war durch eine höhere TFA bei den Behandlungen mit Sojaproteinisolat und durch eine höhere FE bei den Behandlungen mit der freien AS-Mischung bedingt. Entgegen der Versuchshypothese geben diese Ergebnisse keinen Hinweis auf eine unterschiedliche Verwertung von Gly_{equi} für die Harnsäurebildung.

Es kann gefolgert werden, dass die Auswirkungen der Gly_{equi}-Konzentration im Futter von der weiteren Futterbeschaffenheit, wie den Konzentrationen an Cys, Thr, Cholin sowie dem Anteil freier AS abhängt. Die in dieser Dissertation beschriebenen Erkenntnisse ermöglichen eine weitere Optimierung der Gly_{equi}-Konzentration und der weiteren Futterbeschaffenheit, die die Auswirkungen der Gly_{equi}-Konzentration beeinflusst. Dies ermöglicht die XP-Konzentration im Futter ohne nachteilige Auswirkungen auf die Wachstumsleistung zu reduzieren und somit die negativen Auswirkungen der Masthähnchenproduktion auf die Umwelt zu verringern.

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02/2007 – 05/2007	Agricultural internship at the farm of Iris Meyer, Zips/Pegnitz, Germany
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09/2007 – 10/2007	Agricultural internship at the farm of Thomas Einzinger, Reutern/ Wolframs-Eschenbach, Germany
07/2008 – 12/2008	Diploma student for Lallemand Animal Nutrition, Toulouse, France at SAS La Fennetrie, Marçay, France
07/2009 – 03/2011	Student Assistant at Department of Behavioral Physiology of Farm Animals, Department of Food Physics and Meat Science, and Department of Animal Nutrition, University of Hohenheim, Stuttgart, Germany

Hohenheim, 22.10.2015

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Wolfgang Siegert