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Relevance of Glycine in Low Crude Protein Diets for Broilers

Key information

- Glycine and serine can become growth-limiting for 1 to 21 days old broilers in feed containing less than 20 % crude protein.
- Glycine and serine should be assessed together using the glycine equivalent rather than the simple sum of glycerine+serine.
- The requirement for glycine equivalent depends on other nutrients such as threonine, choline, and cysteine. Reversely, the requirement for threonine and choline is not constant, and the optimal ratio between methionine and cysteine depends on the glycine equivalent concentration.
- Adequate glycine equivalent concentrations enable reducing the crude protein concentration in feed for broilers from 1 to 21 days to about 16 % without undesirable effects on growth and feed efficiency.
- Adequate glycine equivalent concentrations in low crude protein feed can only be achieved with using animal proteins and/or free glycine or l-serine.

Introduction

The increasing demand for meat and other animal products along with the global limitation of arable land results in a shortage of protein-rich feedstuff to be expected. Consequences of increased crop prices have been shown to especially affect affordability of food in developing countries.

The excretion of nitrogenous compounds can have negative effects on the environment because the excreted nitrogen (N) can be dispersed into water, soil and air. N contained in manure can be applied as fertilizer, but it risks N leakage into groundwater. Ammonia emissions have been associated with a number of harmful effects on the environment, which include soil acidification, eutrophication, formation of fine particulates, and secondary emissions of nitrous oxide. In addition, high ammonia levels in the animal house can affect human and animal health as well as the acceptance of livestock farming by the public due to unpleasant odors. Ambient temperature and manure characteristics like pH and moisture have an influence on ammonia emissions, but total excretion of N by livestock is most determining (Liu *et al.*, 2007; Namroud *et al.*, 2008).

Excretion of N can be reduced effectively by lowering the crude protein (CP) concentration in feed. Experiments, however, showed that low CP feed may have undesirable effects on performance of farm animals and carcass quality although the requirement of essential amino acids (AA) was met. Various potential reasons have been suggested to explain the undesirable effects of low CP feed (Aftab *et al.*, 2006). The advance in knowledge concerning low CP feed during the past 10 years enables a more precise assessment of potential reasons for undesirable effects. A deficient glycine supply in low CP feed was revealed to be the most substantive.

This article summarizes the outcomes of a PhD project on glycine effects in low CP diets done at the University of Hohenheim, Germany.

Approaches of lowering the crude protein concentration in broiler feed

Different optimal concentration of essential amino acids

It is often discussed that the optimal concentration of essential AA might be different between standard and low CP feed and therefore represents one possible reason for diminished growth when low CP feed is fed to broilers. Low CP feed supplemented with free AA to raise the level of all essential AA above recommendations failed to achieve growth performance as achieved with standard CP feed (e.g. Deschepper and deGroote, 1995; Jiang *et al.*, 2005; Bregendahl *et al.*, 2002). In other studies combinations of essential AA were varied with the result of increasing growth performance partially but without fully overcoming the impaired growth induced by low CP content (e.g. Hussein *et al.*, 2001; Namroud *et al.*, 2008; 2010; Si *et al.*, 2004a;b). Interactive effects of essential AA with other nutrients in the feed reduced along with CP may also have affected growth.

Consideration of nonspecific nonessential amino acids

Effects of nonspecific nonessential AA were investigated by using different combinations or levels of nonessential AA in the feed. Results of these studies were not consistent. To give examples: When added to low CP feed, mixtures of free aspartic acid and glutamic acid (Bregendahl *et al.*, 2002; Leclercq *et al.*, 1994) and a mixture of free aspartic acid, glutamic acid and alanine (Nieß *et al.*, 2003) had no effect on growth performance. However, mixtures of free glutamic acid and glycine (Deschepper and deGroote, 1995; Namroud *et al.*, 2008; 2010), and tyrosine and serine (Thornton *et al.*, 2006) increased performance but not to the level of feed with standard CP concentrations.

Considering nonessential AA as the sum of the concentrations of nonessential AA probably is not sufficient because an unlimited interconversion of nonessential AA is assumed. Results of the aforementioned studies suggest that adequate supply of specific nonessential AA is important in feeding of broilers with low CP diets.

Specific nonessential amino acids

Studies aiming to increase nonessential AA nitrogen by adding free glutamic acid failed to prevent growth depression caused by feeding low CP feed (amongst others Hussein *et al.*, 2001; Kerr and Kidd, 1999). Three studies found that feed with 16 to 18 % CP and supplemented with glycine to the levels of about 22 % CP control feed did not cause differences in growth performance compared to the control feed. The supplementation of other nonessential AA singly did not increase growth performance (Corzo *et al.*, 2005; Dean *et al.*, 2006; Awad *et al.*, 2015). Parr and Summers (1991) also reported no difference in growth performance between feed with 20 % CP supplemented with glycine and feed with 23 % CP and additionally disproved similar effects of free glutamic acid, alanine and aspartic acid. Other studies reported improved growth upon addition of free glycine to low CP feed (amongst others Corzo *et al.*, 2004; Jiang *et al.*, 2005; Schutte *et al.*, 1997).

Since many years the gain to feed ratio (G:F) in broiler production continuously increased. This caused specific nonessential AA becoming more relevant. This can in part be explained by the increasing probability that not enough metabolic precursors for the respective AA are available or because endogenous metabolic processes are too slow (Aftab *et al.*, 2006; Berres *et al.*, 2010). The potential of glycine to increase growth has been known for decades (Almquist and Mecchi, 1940). Since publication of the study of Dean *et al.* (2006), it is broadly accepted that a deficiency of glycine in feed is one factor limiting CP reduction in broiler feed. Glycine was suggested to be the first-limiting nonessential AA (Ospina-Rojas *et al.*, 2012). Waguespack *et al.* (2009) described glycine as the fourth-limiting of all proteinogenic AA after methionine, lysine, and threonine in feed based on corn and soybean meal for broilers from 1 to

18 days. Similarly, Ospina-Rojas *et al.* (2014) described valine and glycine to be equally limiting after methionine, lysine, and threonine in corn-soybean meal-based feed for broilers from 1 to 21 days.

Relationship between glycine and serine

Glycine can be metabolized from serine in a reversible one-step reaction. For poultry, it is generally assumed that the metabolic interconversion of glycine and serine is not limited (Akrabawi and Kratzer, 1968; Sugahara and Kandatsu, 1976). Concentrations of both AA in feed have the same effect on growth performance as long as the same molar quantity is considered. Therefore, glycine and serine usually are assessed together.

As currently common practice, most authors take the analogue effect of glycine and serine as the sum of the concentrations of both AA (Gly+Ser) into account, neglecting that serine only has the same effect as glycine on an equimolar basis. Dean *et al.* (2006) proposed to describe the physiological value by calculating the glycine equivalent (Gly_{equi}) as the sum of the concentration of glycine and the molar Gly_{equi} of serine, calculated as follows:

$$\mathbf{Gly_{equi}\ (g/kg) = glycine\ (g/kg) + [0.7143 \times serine\ (g/kg)]}$$

where 0.7143 is the ratio of the molar weight between glycine and serine. The Gly_{equi} value appears more appropriate than the currently common Gly+Ser value. The advantage of rendering the anticipated growth response more precisely justifies the additional calculation.

Physiological functions of glycine and serine

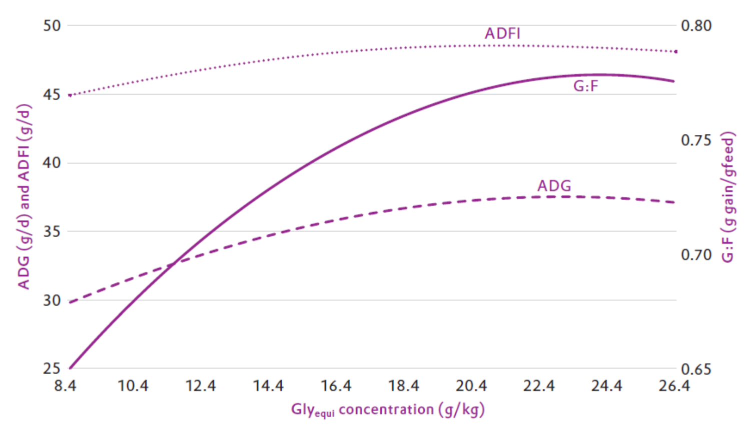
As any other proteinogenic AA, glycine and serine are incorporated into proteins. The proteins richest in glycine are collagen and elastin. Keratin, mainly present in feathers and claws, is rich in both glycine and serine. Mucin proteins are rich in serine. This might explain why a deficiency of glycine and serine caused reduced skin strength due to low collagen content (Christensen *et al.*, 1994), impaired feather development (Robel, 1977), and reduced mucin secretion (Ospina-Rojas *et al.*, 2013). Thus, a deficient supply of Gly_{equi} may have implications in addition to reduced growth performance.

In uricotelic species like chicken, ammonia is detoxified and excreted as uric acid being the main excretion product of N metabolism. The formation of each molecule of uric acid requires one molecule of glycine, which makes glycine more important in poultry than in other farm animals. Glycine is also required as an integral part of creatine along with arginine. Serine is needed for the synthesis of cysteine from methionine.

Response to dietary glycine and serine

Since the importance of glycine and serine in poultry nutrition has been recognized, the number of publications about effects of glycine and serine has increased. Several dose-response studies investigating the effect of glycine and serine were published and summarized in a meta-analysis (Siegert *et al.* 2015a).

Figure 1 Response of ADG, ADFI, and G:F to different concentrations of Gly_{equi} in feed determined in a meta-analysis using 10 published studies that investigated the response of about 1 to 21 days old broilers (Siegert et al., 2015a).

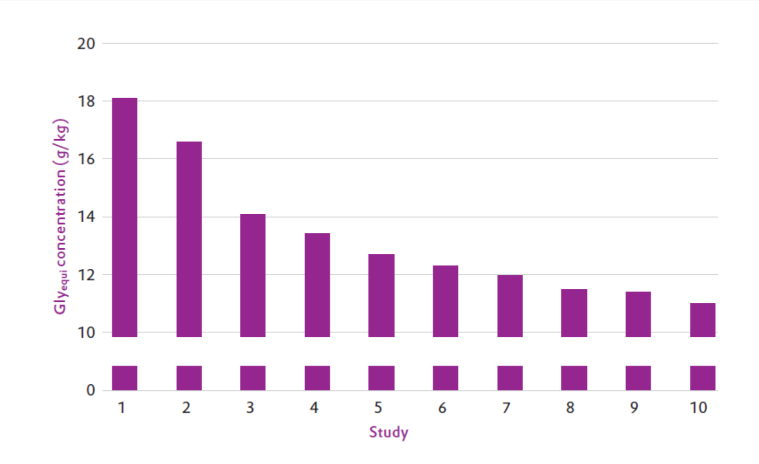


The effect of the Gly_{equi} concentration in feed on the average daily feed intake was low, but the effect on average daily gain (ADG) was more pronounced, and the effect on G:F was most evident (Figure 1). The responses to Gly_{equi} in feed shown in Figure 1 do not consider the particularities of feed that might influence the response to Gly_{equi} in feed. The response to Gly_{equi} in feed, however, varied considerably between studies (Figure 2). Factors influencing the response to Gly_{equi} in low CP feed are addressed in the following chapters.

Effect of cysteine

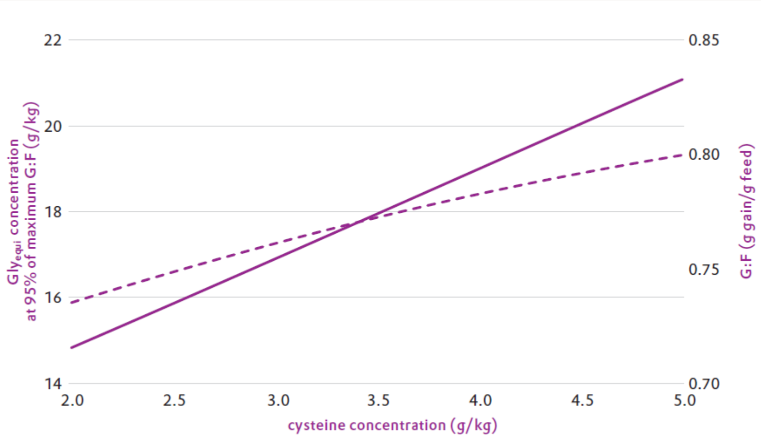
Powell *et al.* (2011) found that the impact of glycine and serine on G:F partly can be explained by the conversion of methionine to cysteine, for which serine is required. They showed an increased G:F after addition of glycine to feed adequate in total sulfur AA (TSAA) but deficient in cysteine. Additional inclusion of methionine was without effect, but the addition of cysteine above the requirement reduced the performance increasing effects of glycine supplementation. This observation is especially important when low CP feed is used because a targeted methionine+cysteine concentration usually is achieved by the addition of dl-methionine disregarding a specific requirement for cysteine.

Figure 2 Gly_{equi} concentration in feed needed to achieve 95 % of the maximum G:F response (own recalculations; based on the 10 studies used by Siegert et al., 2015a).



The meta-analysis by Siegert *et al.* (2015a) showed that the cysteine concentration in feed has a substantial impact on the requirement for Gly_{equi} (Figure 3). Fulfilling the requirement for both methionine and cysteine in broilers reduces the necessity of the conversion of methionine to cysteine. Each molecule of methionine not converted to cysteine reduces the requirement for Gly_{equi}. Despite that, the Gly_{equi} requirement at 95 % of maximum G:F increased with raising cysteine concentrations, but this probably was attributable to an increased Gly_{equi} requirement for protein accretion in consequence of increased growth performance.

Figure 3 Effect of the cysteine concentration in feed on G:F at 95% of maximum response (dashed line) and the required dietary Gly_{equi} concentration at 95% of maximum G:F (solid line) of about 1 to 21 days old (derived from Siegert *et al.*, 2015a).

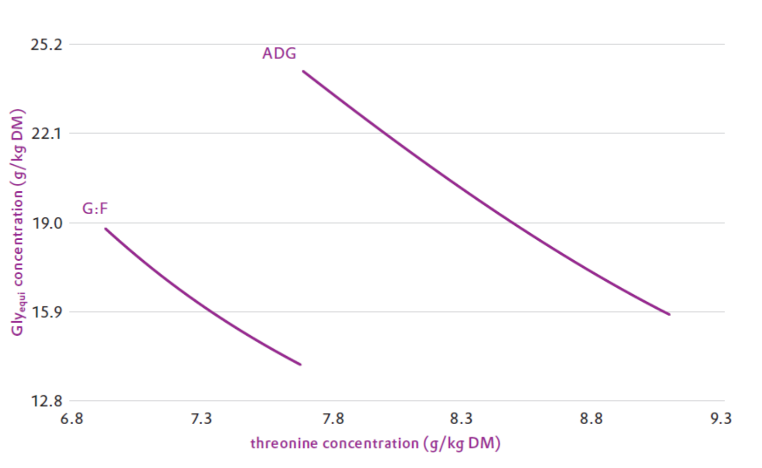


Effect of endogenous precursors of glycine

Several substances can be metabolized to glycine or serine out of which threonine and choline are quantitatively most important. Threonine can be directly metabolized to glycine. Choline, with betaine and dimethylglycine as intermediate steps, is metabolized to glycine if homocysteine is available (Meléndez-Hevia *et al.*, 2009).

The study of Siegert *et al.* (2015b) showed that certain levels of G:F and ADG can be achieved with distinct combinations of Gly_{equi} and threonine (Figure 4). An increase in threonine concentration reduced the Gly_{equi} concentration required to achieve certain response levels. The potential of reducing the Gly_{equi} concentration in feed when the threonine concentration was increased exceeded the theoretically possible replacement explainable by endogenous conversion (e.g. Ospina-Rojas *et al.*, 2013, Siegert *et al.*, 2015b). Consequently, the replacement value of threonine to Gly_{equi} in feed is determined by other factors in addition to endogenous conversion which should be investigated in future investigations.

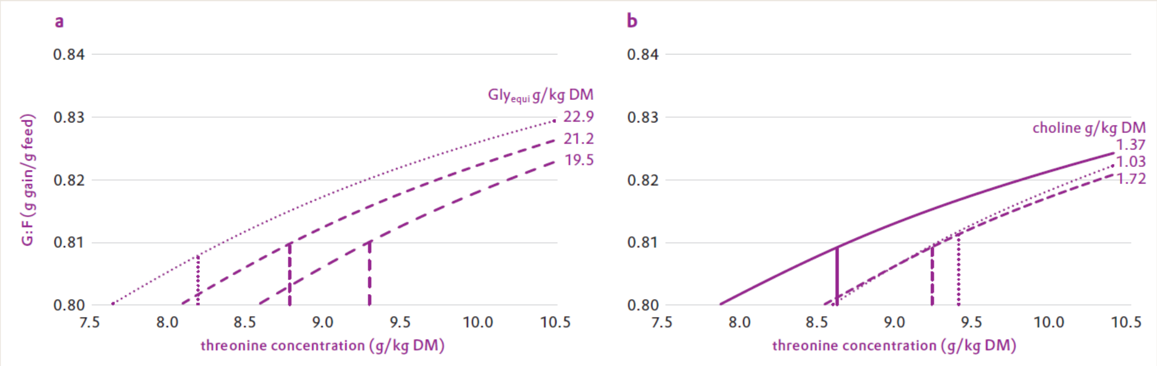
Figure 4 Gly_{equi} concentration in feed necessary to achieve 95% of maximum G:F and ADG responses of 7 to 21 days old broilers depending on the threonine concentration in feed (Siegert *et al.*, 2015b).



Choline can considerably affect the Gly_{equi} concentration required to achieve certain levels of G:F and ADG. The replacement effect of choline for Gly_{equi} is lower than the replacement effect of threonine (Siegert *et al.*, 2015b). The effect of choline depended on the Gly_{equi} and threonine concentration. This shows that the response to one nutrient (out of Gly_{equi}, threonine, and choline) depends on the concentrations of the other nutrients. Therefore, concentrations of Gly_{equi}, threonine, and choline need to be jointly considered in feed formulation. To our knowledge, interactive effects between Gly_{equi} and choline, betaine, or dimethylglycine were not reported in the literature.

The magnitude of mutual replacement effects of Gly_{equi}, threonine, and choline emphasizes the necessity to consider the other nutrients when the requirement of one of those nutrients is derived singly. To give examples: At a fixed choline concentration of 1.37 g/kg dry matter (DM), the threonine requirement at 95 % of maximum G:F was ranged from 8.2 to 9.3 g/kg DM when the Gly_{equi} concentration was varied between 19.5 and 22.9 g/kg DM (Figure 5a). Likewise, at a fixed Gly_{equi} concentration of 19.5 g/kg DM, the threonine requirement at 95 % of maximum G:F ranged from 8.8 to 9.5 g/kg DM when the choline concentration varied between 1.03 and 1.72 g/kg DM (Figure 5b). This might partly explain different results of studies investigating the threonine requirement of broilers (e.g. Kidd *et al.*, 2004; Mehri *et al.*, 2014) and the variable response to choline in feed described by the NRC (1994).

Figure 5 Effect of the threonine concentration in feed on G:F at different concentrations of Gly_{equi} in feed at a fixed choline concentration of 1.05 g/kg DM (a) and the effect of the choline concentration in feed at a fixed Gly_{equi} concentration in feed of 19.5 g/kg DM (b) of 7 to 21 days old broilers based on the results of Siegert *et al.* (2015b). The threonine requirement in feed at 95% of maximum G:F is depicted by the vertical lines±±.



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Effect of creatine formation

Arginine and glycine are precursors of guanidino acetic acid, which is needed to form creatine (Kjajali and Wideman, 2010). Significant interaction effects for combinations of creatine, guanidino acetic acid, and arginine on ADG and G:F of broilers were reported in several studies (e.g. Dilger *et al.*, 2013) but only little information is available about interaction effects between glycine and the aforementioned nutrients in feed. More explanations for different responses to Gly_{equi} might be revealed by studies targeting the effect of precursors of creatine on the Gly_{equi} requirement.

General considerations

As mentioned above, the response to Gly_{equi} depends on the concentrations of cysteine, threonine, and choline and vice versa. The arginine concentration in feed may also have an influence. It appears possible that the growth-increasing effect of supplementing essential AA to low CP feed in some studies (see section "Approaches of lowering the CP concentration in broiler feed") was due to interactions with Gly_{equi}.

Possibilities to vary the Gly_{equi} concentration in feed

The concentration of Gly_{equi} varies considerably between and within types of feedstuffs (Table 1). However, the variation in the proportion of Gly_{equi} in CP is low and ranges between 7.3 and 8.3 g/100g CP in most cereals, cereal byproducts, brewery byproducts, oil seeds, and pulses. The proportion of Gly_{equi} in CP is highest in meat meal and in meat and bone meal.

TABLE 1 Concentrations of Gly_{equi} and proportion of Gly_{equi} in crude protein of selected feedstuffs relevant for animal feeding based on nutrient concentrations extracted from AMINODat® 4.4 (2010).

FEEDSTUFFS		GLY_{EQUI} (G/KG DM)	GLY_{EQUI} (G/100G CP)
Cereals	Winter wheat	8.3	7.4
	Durum	11.1	7.0
	Corn	6.6	7.3
	Triticale	9.5	7.3
	Oats	8.8	7.3
Oil seeds	Rapeseed meal	31.7	7.9
	Soybean meal	41.6	7.8
	Sunflower expeller	44.4	8.3
Pulses	Field beans	21.6	7.4
	Field peas	18.5	7.6
	Lupins	30.4	7.4
Non-animal byproducts	Corn gluten feed	17.1	7.5
	Wheat bran	11.4	8.2
	DDGS[1] (wheat)	22.5	7.2
	Brewer's dried yeast	35.3	7.8
Animal byproducts	Fish meal	64.3	6.4
	Feather meal	130.1	14.7
	Meat meal	11.4	14.8
	Meat and bone meal	14.9	17.7

[1] Distillers dried grains with solubles

Because the variation of Gly_{equi} in CP is low, using only vegetable raw materials in feed formulation causes the Gly_{equi} concentration in feed to depend mainly on the CP concentration. It is hardly possible to increase or decrease the proportion of Gly_{equi} in CP of plant-based feed. Using animal byproducts elevates the Gly_{equi} concentration in feed but feeding of animal-derived proteins to poultry currently is prohibited in some parts of the world. Feed additives suitable for elevating the Gly_{equi} concentration in feed are free glycine and l-serine, which are not approved in some countries. It is hardly possible to achieve adequate Gly_{equi} concentrations in low CP feed in countries where animal byproducts in broiler feed are prohibited and the use of free glycine and l-serine is not approved.

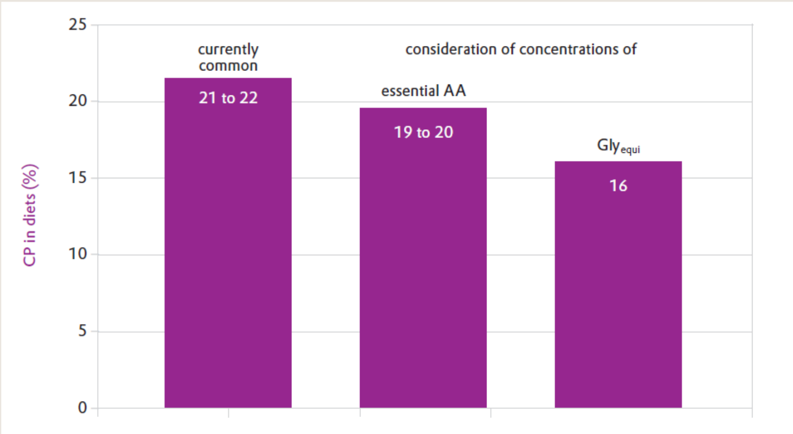
A deficiency in Gly_{equi} can be reduced by a surplus of endogenous precursors like threonine and choline. Betaine and dimethylglycine as intermediate steps of the glycine formation from choline might also be suitable endogenous precursors, but no literature is available on that.

Current status and perspectives of crude protein reduction

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. Influences on the lower limit of CP concentration, like period of growth, sex, criterion of response or health status are numerous. The complexity of the many influencing factors on the requirement for AA suggests that future research can further approach but probably not reach the ideal AA composition of feed by diminishing safety margins.

Current research mostly aims to find ways to reduce the CP concentration in feed without compromising the level of ADG and G:F achieved nowadays with common CP concentrations (e.g. Corzo *et al.*, 2005; Dean *et al.*, 2006). This is at about 21 to 22 % CP in diets for broilers from 1 to 21 days post-hatch (Figure 6). Dean *et al.* (2006) summarized that ADG and G:F of broilers fed with diets containing less than 19 to 20 % CP was reduced even when the requirement for essential AA was met. If the Gly_{equi} concentrations was adequate, ADG and G:F of broilers fed diets containing 16 to 17 % CP were at the level of diets containing more than 20 % CP (Figure 6) (Corzo *et al.*, 2004, Dean *et al.*, 2006; Siegert *et al.*, 2015a).

Figure 6 Currently common CP concentrations in feed for 1 to 21 days old broilers and estimations to which level the CP concentration in feed might be reduced without affecting growth performance by additional consideration of other nutrient concentrations in feed.



Factors influencing the response to Gly_{equi} in feed probably were suboptimal in the studies mentioned before. Consequently, further reduction of the CP concentration in feed without negative effects on growth performance should be possible through optimization of both the Gly_{equi} concentration in feed and factors influencing the response to Gly_{equi}.

Glycine and serine are the first nonessential AA of which experimentally verified requirement values were reported. To our knowledge, such values are not available for other nonessential AA. The possibility to further reduce the CP concentration in feed without adverse effects on performance can be expected when the role of other nonessential AA is better understood, and experimentally verified requirement values are available.

Conclusions

Different concentrations of essential AA or the ratio between the sums of essential AA to nonessential AA do not allow to reduce the CP concentration considerably below 20 % in the feed of 1-21 days old broilers without adverse effects on performance. Gly_{equi} becomes growth-limiting in feed with a CP concentration below 20 %. Consideration of Gly_{equi} in feed enables to reduce the CP concentration in broiler diets remarkably. The requirement for Gly_{equi} depends on the concentrations of other nutrients in the feed such as threonine, choline, and cysteine. Reversely, the requirement for threonine and choline is not constant but depends on Gly_{equi}. An optimized methionine:cysteine ratio also depends on the Gly_{equi} concentration in the feed.

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Acronyms

AA	amino acids
ADG	average daily gain
CP	crude protein
DM	dry matter
G:F	gain to feed ratio
Gly_{equi}	glycine equivalent
Gly+Ser	glycine+serine
N	nitrogen
TSAA	total sulfur amino acids

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