

Review Article

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AA, Amino acid; CP, Crude protein; Gly_{equi}, Glycine equivalents; ME_N, Nitrogen-corrected metabolisable energy; N, Nitrogen; NUE, Nitrogen utilisation efficiency

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Relevance of nonessential amino acids in low crude protein diets for broiler chickens – an updated review

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Abstract

Reducing crude protein in amino acid-adequate diets for broiler chickens is effective in reducing nitrogenous emissions and competition for resources between the food and feed sectors. This review provides a comprehensive analysis of the literature on the relevance of nonessential amino acids in low protein diets for broiler chickens. Glycine and serine, owing to their interconvertibility summarised as glycine equivalents (Gly_{equi}), limit growth when dietary crude protein is reduced below 19% in up to 3-week-old birds. Considering essential amino acids and the variable Gly_{equi} requirements enables the reduction of dietary crude protein to ~16% without compromising growth. Variation in Gly_{equi} requirements likely occurs predominantly from the varying amounts of uric acid formed. Other influences seem to exert lower impacts on dietary Gly_{equi} requirements. Asparagine or glutamine is probably the growth-limiting amino acid when crude protein is reduced below 16%. Alternatively, nonspecific amino-nitrogen may be lacking in such diets. The current potential to reduce dietary crude protein when using free essential and nonessential amino acids enables to increase the efficiency of nitrogen utilisation to a value above 80%. This coincides with reduced uric acid synthesis and energy expenditure for nitrogen excretion. The lower nitrogen excretion via the urine results in a lower energy expenditure. Hence, dietary energy may prospectively be reduced once the energy-sparing effect is quantified, thereby further reducing the competition for resources between food and feed.

Introduction

Poultry meat is the most common animal-based food worldwide, followed by pork, beef and sheep meat. Increasing meat consumption is expected for all of the mentioned meat types in the next decades, with poultry meat expected to increase most⁽¹⁾. Production of animal-based food requires land and water, mainly for the production of feed crops⁽²⁾. Hence, arable land and water for cropping are proposed to become an increasingly scarce resource as the world population increases. Another aspect is the environmental impact of animal farming. Major environmentally relevant impacts of feed production include energy consumption of cropping and transport, carbon footprints of feed production including free amino acids (AA), emissions from the fields and the consequences of land-use change when crops are cultivated on converted forests or grasslands. Regarding manure, the major environmental impact includes nitrogenous emissions, such as ammonia, nitrate and nitrous oxide. These emissions contribute to climate change, acidification, eutrophication and air and water pollution⁽³⁾.

Aims of farm animal nutrition research include minimising the negative effects of the livestock sector on the environment and contributing to global food security. These goals can be approached by reducing the dietary crude protein (CP) concentration in animal diets without undesirable effects such as health implications and reduced performance. This increases the efficiency of the conversion of protein from the feed to animal protein ('nitrogen (N) utilisation efficiency (NUE)') and reduces the excretion of nitrogenous compounds and the use of protein-rich feed ingredients such as soya products. Investigations enabling the precise supply of AA and other nitrogenous nutrients according to their situation-specific requirements contribute to decreasing dietary CP below current standards. Such investigations include studies on the requirements of the animals and on the nutrient digestibility of feed ingredients and compound diets⁽⁴⁾. In terms of CP reduction, knowledge on requirements for AA is primarily relevant. Such investigations have enabled a considerable reduction in CP concentration in poultry feed and have reduced N excretion substantially^(5,6). Such CP reductions mostly represent a decrease in surpluses because lack of knowledge or variability within a flock is countered by safety margins in AA concentrations if the aim is to exclude a risk of AA deficiency.

Particularly in the past decade, the increase in knowledge on AA requirements has revealed a big potential to reduce dietary CP from 19–20% to ~16% in diets for broiler chickens⁽⁵⁾. This was made possible because the limiting role in CP reduction of the nonessential AA glycine (Gly) and serine (Ser) was realised, mainly owing to studies that were published in the mid-2000s^(7,8). Nonessential AA can limit protein biosynthesis if the provision by the feed and endogenous production are insufficient, for instance, due to a lack of precursors, or if endogenous processes are too slow⁽⁸⁾.

A review from 2019 summarised the knowledge on responses of poultry to the dietary supply of Gly and Ser of the time⁽⁵⁾. Since then, the knowledge on Gly, Ser and other relevant nonessential AA has advanced considerably. The objective of this review is to update the information presented in the previous review⁽⁵⁾ and evaluate the relevance of other nonessential AA for the goal of reducing CP in diets for broiler chickens. This includes inferences on the energy requirement of the animals, which is also affected by the dietary CP concentration. Published studies on effects of nonessential AA mostly investigated common fast-growing broiler strains; hence, effects of dietary nutrient concentrations may differ for other strains.

How to consider similar effects of dietary glycine and serine

Gly and Ser are assessed together in diet formulation because Gly and Ser are metabolically interconvertible⁽⁸⁾. Early recommendations for dietary nutrient composition accounted for this relationship using 'Gly+Ser', where dietary Gly and Ser concentrations are added together⁽⁹⁾. This sum assumes that Ser is metabolically equivalent to Gly on a mass basis. However, the ratio of the molar weight of Gly to Ser is 0.714^(10,11), mainly reflecting the additional hydroxymethyl group of Ser⁽¹²⁾. This means that more Gly and Ser molecules are provided to the animals the more Gly is contained in Gly+Ser. Dean *et al.*⁽⁸⁾ suggested the reference unit 'Gly equivalent' (Gly_{equi}) as the sum of Gly and the molar Gly equivalent of Ser, calculated as Gly_{equi} (g/kg) = Gly (g/kg) + 0.714 × Ser (g/kg). The practical relevance of the increased accuracy of Gly_{equi} compared with Gly+Ser is probably low in purely plant-based diets for poultry. Reasons include the roughly even share of Gly:Ser on a mass basis in most plant-based feed ingredients, with shares in the range of 0.45:0.55 to 0.60:0.40⁽⁵⁾. However, the share of Gly:Ser can deviate considerably from these ranges (0.30:0.70 to 0.85:0.15) when animal by-products are used⁽⁵⁾. In addition, it is more likely that free Gly is supplemented to the diets, as free L-Ser is more expensive than free Gly. The influence of free Gly supplementation on the Gly:Ser share can be considerable. As an example, using free Gly to maintain a dietary Gly_{equi} concentration of 15 g/kg when dietary CP was reduced from 16.3% to 13.2% resulted in Gly:Ser shares increasing from 0.54:0.46 to 0.75:0.25⁽¹³⁾. Therefore, using Gly_{equi} as a reference unit appears more advisable than using Gly+Ser.

Recently, the concept of an equal utilisation of Gly and Ser was challenged⁽¹⁴⁾. This study reported that Gly_{equi} is more precise than Gly+Ser in representing the physiological value of these AA in the diet. The conditions under which Gly and Ser are not equally utilised were theoretically derived based on extensive stoichiometric calculations of metabolic pathways. The key point was that one more one-carbon unit can be generated from Ser than from Gly metabolism and Gly can take up a one-carbon unit. In poultry,

uric acid formation is one way to counteract the accumulation of one-carbon units. Uric acid formation consumes both Gly and one-carbon units. Hence, Gly is needed to prevent the accumulation of one-carbon units, while Ser promotes this accumulation. Provided that there are no other physiological adaptations that prevent an accumulation of one-carbon units, less Gly would be available for other metabolic functions, including protein synthesis. The actual relevance of a possible accumulation of one-carbon units has not yet been experimentally investigated. Therefore, this review continues using Gly_{equi}.

Relevance of influences on the Gly_{equi} requirements

Estimates of the adequate dietary Gly_{equi} concentration differed widely among studies with values ranging from below 11 g/kg to more than 18 g/kg of diet⁽⁵⁾. Several factors influencing the Gly_{equi} requirement were determined.

Influence of nitrogen excretion

The adjustment of the supply to the requirement for digestible AA seems to be the major determinant of the Gly_{equi} requirement. The physiological background is that AA that cannot be used for metabolic functions are oxidised and the contained N is excreted via the urine, mostly as uric acid⁽¹⁵⁾. Uric acid formation is a Gly-dissipating process because each uric acid molecule requires one molecule of Gly to build the purine ring when glycinamide ribotide is synthesised from phosphoribosylamine⁽¹⁶⁾. A model calculation of Siegert and Rodehutschord⁽⁵⁾ suggested that uric acid formation is a major contributor to variable Gly_{equi} requirements. Selle *et al.*⁽¹⁷⁾ quantified the proportion of Gly intake relative to uric acid output in the range of 25–81% based on experimental data. Recalculations of data from our own published studies showed lower proportions of Gly_{equi} intake relative to uric acid excretion (both mol/d) with ranges of 8–30%⁽¹³⁾ and 9–19%⁽¹⁸⁾. Nonetheless, these calculations suggest a considerable share of dietary Gly and Ser being used for uric acid synthesis.

Pursuing the goal of a higher NUE reduces the Gly_{equi} requirement of broiler chickens because less N is excreted as uric acid. This was theoretically derived in a model calculation of Siegert and Rodehutschord⁽⁵⁾ and is substantiated by data from animal experiments (Fig. 1), which show that the Gly or Gly_{equi} intake used for uric acid excretion decreased considerably with increasing NUE. The decreasing Gly_{equi} requirement with increasing NUE indicates that supplementing free Gly or L-Ser to diets very low in CP may become irrelevant in the future provided that CP in AA-adequate diets can be considerably reduced beyond the current potential. Considering dietary Gly_{equi} in diet formulation would become unnecessary if the goal of increasing the NUE results in an amount uric acid formation that is low enough so that the Gly_{equi} supply from plant-based feed meets or exceeds the requirement of the birds.

Further influences

There are other influences on the Gly_{equi} requirement, but the extent of their impact probably is low compared with uric acid formation. Such influences include dietary threonine and choline concentrations because Gly or Ser can be formed in the metabolism of the animals from these compounds⁽¹⁹⁾. The ratio between methionine and methionine+cysteine (Met:(Met+Cys)) also impacts the Gly_{equi} requirement due to the endogenous conversion of methionine to cysteine, which

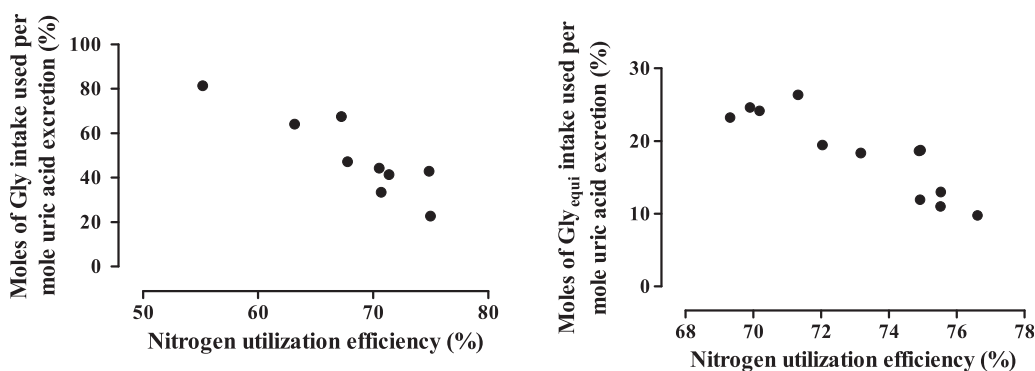


Fig. 1. Proportion of Gly intake per uric acid output (left panel: results of an experiment published by Chrystal *et al.*⁽⁶⁹⁾ and Selle *et al.*⁽¹⁷⁾; dots represent least square means; $n = 6$) or Gly_{equi} intake per uric acid output (right panel: Hofmann *et al.*⁽¹³⁾; dots represent least square means; $n = 7$) and the nitrogen utilisation efficiency in studies on broiler chickens.

dissipates Ser⁽¹⁹⁾. Studies have shown that the impacts of threonine, choline and the Met:(Met+Cys) ratio on the Gly_{equi} requirement are inconsistent (Table 1 and Table 2). Some studies found a marked impact of the mentioned factors on performance responses of broiler chickens to dietary Gly_{equi}^(e.g. 20–23), while others reported minor⁽²⁴⁾ or no impact^(18,25). One possible contributor to the absence of interaction effects of dietary threonine and Gly_{equi} on performance in studies^(24,25) is a relatively low variation in dietary threonine and Gly_{equi} supply and an overall high Gly_{equi} supply. These characteristics may have made interaction effects less likely to occur. However, variation of dietary Gly_{equi} and the Met:(Met+Cys) ratio was considerable in another study⁽¹⁸⁾, but resulted in interactions of performance traits to only a low extent. The NUE was very high, at approximately 80%, and the variation in NUE was small in the study, with a high variation of dietary Gly_{equi} and the Met:(Met+Cys) ratio⁽¹⁸⁾. This probably resulted in a low Gly_{equi} requirement of the broiler chickens that was barely influenced by uric acid formation. The marked impact of interactions between dietary Gly_{equi} and the other mentioned factors most likely represented a consequence of several influences. The endogenous formation of Gly_{equi} from threonine and choline, or the Gly_{equi} dissipation when methionine was converted to cysteine, probably was one influence. Another probable influence was the impact of the varying dietary nutrient concentrations on NUE, which leads to variable uric acid formation. However, most of the mentioned studies did not report NUE or uric acid formation. Given the big impact of NUE/uric acid formation on the Gly_{equi} requirement^(5,17), the small interaction effects in the study with a high variation of dietary Gly_{equi} and the Met:(Met+Cys) ratio⁽¹⁸⁾, where NUE was almost unaffected, suggest that impacts on Gly_{equi} requirements other than NUE/uric acid formation have little impact.

The hypothesised minor influence of other impacts on Gly_{equi} requirements than NUE/uric acid formation appears beneficial for future consideration of the variable Gly_{equi} requirement when practical diets are formulated. Although desirable for even higher precision, modelling the numerous impacts of dietary threonine, Met, cysteine, and choline – and their interdependencies on Gly_{equi} requirements – seems cumbersome for current practical diet formulation. Possibilities of more precise modelling of metabolic processes may change this. However, considering NUE/uric acid formation as the major contributor to variable Gly_{equi} requirements may be manageable in the near future.

Varying collagen and elastin formation may additionally determine the Gly_{equi} requirement because Gly makes up a considerable share of the AA in collagen and elastin^(19,26). We are not aware of studies investigating the relationship between Gly_{equi} supply and the accretion of collagen and elastin. However,

influences of the Gly supply on collagen content in the skin was described for pigs^(27,28). Possibly, Gly requirements for collagen formation contributed to effects of supplemented Gly on the performance of pigs⁽²⁹⁾, although Gly usage for uric acid formation in pigs is low compared with poultry.

Limitations of nonessential amino acids in Gly_{equi}-adequate diets

Hofmann *et al.*⁽¹³⁾ found high growth performance of 8–21-day-old broiler chickens with 16.3% CP in the diet and an adequate supply of essential AA and Gly_{equi}. However, further dietary CP reduction to 14.7% reduced growth in that study. Hence, other nonessential AA became growth-limiting when CP was reduced from 16.3% to 14.7%. Consistently, growth performance of 14–35-day-old broiler chickens was not reduced when the CP concentration in Gly-supplemented diets was lowered to 16.5% (representing the lowest CP concentration applied) in the study by Chrystal *et al.*⁽²⁴⁾.

Effects of nonessential AA other than Gly and Ser have been studied in diets with growth-limiting nonessential AA concentrations. This includes supplementation of single nonessential AA such as glutamic acid (Glu), glutamine (Gln), aspartic acid (Asp), asparagine (Asn), alanine, and proline and mixtures of two or more of these AA^(e.g. 30–33). Minor or no effects on growth performance were reported in those studies. The composition of the investigated diets suggests that the supply of Gly_{equi} was growth-limiting in those studies. This may explain the absence of any AA supply effect.

The next limiting factor in low CP diets adequate in Gly_{equi} is currently unknown. While the character of Gly_{equi} as the first limiting nonessential AA mostly is independent of the used feed ingredients, because the ratio between Gly_{equi} and CP is similar among plant-based feed ingredients⁽⁵⁾, the proportion of other nonessential AA in CP differs considerably among feed ingredients. Hence, the next limiting nonessential AA probably depends on feed ingredients and dietary CP. In principle, any of the remaining nonessential AA or nonspecific amino-N could be next-limiting. Recent investigations, as detailed in the following subsections, point to Asn, Gln or nonspecific amino-N from nonessential AA other than Gly and Ser. Advancements in the field are complicated by difficulties associated with the chemical analysis of Asn and Gln because hydrolysis of proteins prior to AA quantification leads to Asn and Gln losing an amide residue. Hence, Asn and Gln elute together with Asp and Glu, respectively⁽³⁴⁾. Nonetheless, other nonessential AA can have specific effects, such as an increased feed intake in alanine-supplemented diets⁽³⁵⁾.

Table 1. Summary of studies investigating interactions between dietary Thr and dietary Gly_{equi} on growth performance and other selected response traits by varying nutrient concentrations using free glycine, L-serine and L-Thr. A study reporting three-way interactions of dietary Gly_{equi}, Thr and choline is also included

Study ²	Age range	Investigated response traits	Crude protein levels in the basal diets	Concentrations of varied nutrients	Main outcomes
Waldroup <i>et al.</i> ⁽⁷²⁾	d 0–21	Growth performance	16.4%, 18.6%, 20.7%, 22.7%, 24.3% ^{3,4}	Gly _{equi} ^{4,5} : 19.6, 17.0, 15.6, 13.7, 11.6 g/kg in the basal diets differing in crude protein, supplemented with 0, 2.0, 4.0 g/kg as free Gly Thr ⁴ : 9.7, 8.6, 7.8, 7.7, 7.6 g/kg in the basal diets differing in crude protein, supplemented with 0, 2.0, 4.0 g L-Thr/kg	<ul style="list-style-type: none"> Gly supplementation increased weight gain and numerically increased gain:feed ratio at a crude protein level of 18.6% or lower Dietary crude protein below 22.7% reduced performance No effect of Thr supplementation Overall: Gly_{equi} was a limiting factor in low crude protein diets while Thr had no effect
Corzo <i>et al.</i> ⁽²⁰⁾	d 21–42	Growth performance, carcass traits, breast meat weight, blood plasma	18.3% ^{2,3}	Gly _{equi} ^{4,5} : 13.9, 14.8 g/kg Thr ⁴ : 7.2, 7.7, 8.1 g/kg	<ul style="list-style-type: none"> Increasing Gly_{equi} increased performance traits and carcass weights at 7.2 g Thr/kg but not at 7.7 and 8.1 g Thr/kg In blood plasma, free Gly and Ser increased upon Gly_{equi} supplementation, free Thr increased upon Thr supplementation; no further amino acids reported Overall: Gly_{equi} only was a limiting factor when Thr was low
Ospina-Rojas <i>et al.</i> ⁽⁶³⁾	d 1–7 and d 1–21	Growth performance, breast weight	19.5% ^{3,4}	Gly+Ser ^{4,6} : 18.0, 20.1, 21.0, 22.5 g/kg Thr ⁴ : 9.4, 10.9 g/kg, corresponding to 100 and 115% of the recommendations, respectively	<ul style="list-style-type: none"> On d 1–7, increasing Gly+Ser increased performance at 9.4 g Thr/kg and decreased performance at 10.9 g Thr/kg On d 1–21, increasing dietary Thr decreased weight gain while Gly+Ser had no effect; the gain:feed ratio increased with Gly+Ser at low dietary Thr while Gly+Ser had no effect at high dietary Thr Breast weight increased with Gly+Ser, increasing Thr decreased breast weight Overall: Gly+Ser only was a limiting factor when Thr was low
Ospina-Rojas <i>et al.</i> ⁽⁷³⁾	d 21–35	Growth performance, mucin secretion	17.0% ^{3,4}	Gly+Ser ^{4,6} : 14.4, 16.1, 17.1, 17.6 g/kg Thr: 8.4, 9.2 g/kg, corresponding to 100 and 115% of the recommendations, respectively	<ul style="list-style-type: none"> Increasing Gly+Ser increased weight gain while Thr had no effect Increasing Gly+Ser increased the gain:feed ratio at low Thr while Gly+Ser had no effect at high Thr Increasing Gly+Ser and Thr increased mucin secretion independently Overall: Gly+Ser only was a limiting factor when Thr was low
Siebert <i>et al.</i> ⁽²²⁾	d 7–21	Growth performance	18.0% ^{4,7} (arithmetic mean of fifteen diets)	Gly _{equi} ^{4,7} : 13.2, 15.4, 17.6, 19.8, 22.0 g/kg Thr ^{4,7} : 5.6, 6.5, 7.4, 8.3, 9.2 g/kg Choline ⁷ : 0.5, 0.8, 1.1, 1.5, 1.8 g/kg (calculated) fractional central composite design	<ul style="list-style-type: none"> Increasing Thr decreased the needed Gly_{equi} to achieve defined levels of weight gain and the gain:feed ratio; replacement values were linear for weight gain, while replacement values were higher the higher the gain:feed ratio was Choline influenced replacement values of Gly_{equi} to Thr, with bigger effects the lower the performance levels were; increasing choline in most cases decreased performance Overall: Gly_{equi} effects were more pronounced when Thr was low, choline effects were small and more pronounced the lower Gly_{equi} and Thr was
Hilliar <i>et al.</i> ⁽²⁵⁾	d 7–21 d 21–35	Growth performance, relative breast weight, blood plasma	d 7–21 control and basal: 22.3% and 18.6%, respectively ^{3,4} d 21–35 control and basal: 19.9% and 17.2%, respectively ^{3,4}	d 7–21: Gly _{equi} ^{3,4} : 19.0 g/kg (control), 13.4 (basal), 16.0 and 16.5 g/kg with supplementation of free Gly and free L-Ser, respectively Thr ^{3,4} : 8.6 g/kg (control), 7.4, 12.2 g/kg (low CP diets) d 21–35: Gly _{equi} ^{3,4} : 15.5 g/kg (control), 12.0 (basal), 14.5 and 14.6 g/kg with supplementation of free Gly and free L-Ser, respectively Thr ^{2,3} : 9.4 g/kg (control), 7.2, 11.1 g/kg (low CP diets)	<ul style="list-style-type: none"> No treatment effect on weight gain CP reduction reduced the gain:feed ratio No effect on the gain:feed ratio upon supplementing free Gly, L-Ser, and L-Thr No effect of CP reduction on relative breast weight, supplementing free Gly, L-Ser, and L-Thr reduced relative breast weight In blood plasma, CP reduction reduced free Gly but not free Thr and Ser; L-Thr supplementation increased plasma Thr only; Gly supplementation raised Gly, Ser, and Thr; L-Ser supplementation increased Ser and Thr; other amino acids influenced by CP reduction but not by amino acid supplementation Overall: Gly_{equi} and Thr likely no limiting factors for performance

Table 1. (Continued)

Chrystal <i>et al.</i> ⁽²⁴⁾	d 7–35	Growth performance, blood plasma	16.5% ^{3,4}	Gly _{equi} ⁴ : 10.2 and 14.1 g/kg Thr ⁴ : 7.2 and 8.1 g/kg	<ul style="list-style-type: none"> • No effect on performance • Reduced relative fat pad weight at high Thr • In blood plasma, higher dietary Thr increased Thr but not when Gly_{equi} was also high; higher dietary Gly_{equi} raised Gly, Ser only increased when dietary Thr also high; Arg increased by both high dietary Gly_{equi} and Thr; His and Gln decreased by high Gly_{equi} but not when dietary Thr was high; further amino acids not influenced • Overall: Gly_{equi} and Thr likely not limiting factors for performance
Star <i>et al.</i> ⁽⁷⁴⁾	d 7–28	Growth performance	18.6% ^{3,4}	Gly _{equi} ^{4,5} : 13.9, 14.5, 16.3, 17.0 g/kg (arithmetic means of four diets each) Thr ⁴ : 6.8, 7.0, 7.2, 7.6 g/kg (arithmetic means of four diets each)	<ul style="list-style-type: none"> • Reduced weight gain for lowest dietary Gly_{equi} and Thr, increasing dietary Thr reduced weight gain at 16.3 g Gly_{equi}/kg and higher • Lowest dietary Gly_{equi} reduced the gain:feed ratio at all dietary Thr levels, no increase in the gain:feed ratio at 16.3 g Gly_{equi}/kg and higher • Overall: Gly_{equi} only was a limiting factor when Thr was low
Aguihe <i>et al.</i> ⁽⁷⁵⁾	d 1–21	Growth performance, carcass traits	17.4% ^{3,4}	Gly _{equi} ^{4,5} : 15.4, 16.9, 18.4, 21.4 g/kg (possibly by 2.6 g/kg lower because presented total and digestible amino acid concentrations are identical) Thr ⁴ : 7.6, 8.8, 10.0 g/kg corresponding to 85, 100 and 115% of the recommendations, respectively	<ul style="list-style-type: none"> • Dietary Gly_{equi} increased weight gain up to 16.9 g/kg with no further increases at higher dietary Gly_{equi}; no effect of dietary Thr on weight gain • Increasing dietary Gly_{equi} increased the gain:feed ratio throughout the investigated range for 7.6 and 8.8 g Thr/kg while increasing dietary Gly_{equi} to 18.4 g/kg and more decreased the gain:feed ratio at 10.0 g Thr/kg; the gain:feed ratio was increased from 7.6 to 8.8 g Thr/kg but was decreased at higher dietary Thr • No effects on relative carcass weights • Overall: Gly_{equi} was a limiting factor, high Thr reduced performance at high Gly_{equi} levels

¹Gly_{equi}, glycine equivalents; Gly+Ser, glycine+serine; Ser, serine; Thr, threonine

²Sorted by publication year and alphabetically within publication years

³On an as-fed basis

⁴Analysed values presented

⁵Converted from Gly+Ser to Gly_{equi} on the basis of presented values of Gly and Ser

⁶Gly_{equi} not computable because Gly and Ser were not reported separately

⁷Presented on a standardised dry matter of 88%

Table 2. Summary of studies investigating interactions between the dietary Met to the sum of Met and Cys ratio (Met:(Met+Cys)) and dietary Gly_{equi} on growth performance and other selected response traits by varying nutrient concentrations using free Gly, Cys and Met. A study reporting three-way interactions of dietary Gly_{equi}, Met:(Met+Cys) and choline is also included

Study ²	Age range	Investigated response traits	Crude protein levels in the basal diets	Concentrations of varied nutrients	Main outcomes
Powell <i>et al.</i> ⁽⁷⁶⁾	d 0–18	Growth performance	21.1% ^{3,4}	Both experiments: Gly+Ser ^{3,4,5} : 19.5, 23.2 g/kg Experiment 1 ^{3,4} : Met:Cys ratios: 60:40 (5.7 g Met/kg, 3.5 g Cys/kg) 50:50 (4.9 g Met/kg, 4.2 g Cys/kg) 40:60 (4.0 g Met/kg, 4.9 g Cys/kg) 50:50 (5.3 g Met/kg, 4.6 g Cys/kg) Experiment 2 ^{3,4} : 3.4 g Met/kg and 3.5, 4.0, 4.6, 5.0, 5.6 g Cys/kg	<ul style="list-style-type: none"> Gly supplementation increased the gain:feed ratio, with bigger effects at Met and Cys levels that limited performance An increasing effect of Gly on the gain:feed ratio diminished as dietary Cys levels approached or exceed recommended levels No treatment effects on weight gain Overall: Gly_{equi} only was a limiting factor when Cys was low
Hofmann <i>et al.</i> ⁽¹⁸⁾	d 7–22	Growth performance, nitrogen utilisation efficiency	17.8% ^{4,6} (arithmetic mean of fifteen diets)	Gly _{equi} ^{4,6} : 9.1, 12.1, 15.0, 18.3, 21.3 g/kg Met+Cys ^{4,6} : 8.5 with Cys contribution of 1.8, 2.7, 3.5, 4.4, 5.1 g/kg Choline ⁶ : 0.5, 0.8, 1.1, 1.4, 1.8 g/kg fractional central composite design	<ul style="list-style-type: none"> At medium values of other varied nutrients, increase in the gain:feed ratio up to 13 g Gly_{equi}/kg, and no big effects of higher Gly_{equi} levels Small gain:feed ratio effects of additional Gly_{equi} at high Met:(Met+Cys) from 12 g/kg and at low Met:(Met+Cys) from 14 g/kg, with a higher response in the range of 12 to 15 g Gly_{equi}/kg at low Met:(Met+Cys), suggesting an interaction Negligible impacts of choline on the gain:feed ratio No treatment effects on weight gain Increasing nitrogen utilisation efficiency up to 14.5 g Gly_{equi}/kg, decreasing values at higher Gly_{equi}, with slight deviations from this Gly_{equi} value for varying Met:(Met+Cys) values, suggesting an interaction Overall: Small extent of interactions between Gly_{equi} and Met:(Met+Cys) in a small range of dietary Gly_{equi}; choline effects negligible
Aguihe <i>et al.</i> ⁽⁷⁷⁾	d 1–21	Growth performance, relative breast meat yield	18.1% ^{3,4}	Gly _{equi} ^{3,4} : 14.9, 16.4, 17.9, 19.4, 20.6 g/kg Met+Cys ^{3,4} : 8.7, 10.0, 11.3 g/kg through addition of free Met	<ul style="list-style-type: none"> No interaction between Gly_{equi} and Met+Cys on growth performance Highest performance at medium Met+Cys values Performance increased up to 19.4 g Gly_{equi}/kg, with no changes at higher Gly_{equi} values Relative breast meat yield increased at low Met+Cys through the investigated Gly_{equi} range, while Gly_{equi} increased relative breast meat yield at medium and high Met+Cys up to 19.4 g Gly_{equi}/kg, with decreasing values at higher Gly_{equi}; relative breast meat yield was lowest at high Met+Cys, particularly at high Gly_{equi} Overall: Gly_{equi} and Met+Cys (and consequently the Met:(Met+Cys) ratio) not limiting factors for growth performance, Gly_{equi} apparently a limiting factor for breast meat yield at low Met+Cys

¹Met, methionine; Cys, cysteine; Gly_{equi}, glycine equivalents

²Sorted by publication year

³On an as-fed basis

⁴Analysed values presented

⁵Gly_{equi} not computable because Gly and Ser were not reported separately

⁶Presented on a standardised dry matter of 88%

Indications of asparagine being the next-limiting nonessential amino acid

A study identified Asn as the most likely candidate for the next-limiting nonessential AA⁽³⁶⁾. In that study, the supplementation of alanine and proline to low CP diets adequate in Gly_{equi} had no effect on growth performance traits, but supplementation of Asn+Asp, Glu and Gln+Glu increased growth compared with the unsupplemented diet. Responses in growth were not different among diets supplemented with Glu or 50:50 mixtures of Asn+Asp and Gln+Glu. The similar effects of Asn+Asp, Glu and Gln+Glu supplementation might be explained by the few steps needed for the metabolic interconversion of those AA⁽³⁷⁾. However, growth performance was higher when Asn+Asp was supplemented than with Asp supplementation alone.

A recent study by Ibrahim *et al.*⁽³⁸⁾ further substantiated the probability that Asn is the next-limiting nonessential AA. In that study, digestible peptide-bound AA from soya protein isolate were incrementally substituted with free AA so that the digestible AA concentration was identical in all diets. The analysed digestible concentrations of Asn/Asp and Gln/Glu were either substituted by Asp and Glu, respectively, or by 50:50 mixtures of Asn+Asp and Gln+Glu, respectively. Growth performance declined at a certain substitution of peptide-bound with free AA. This decline was determined at higher substitutions when Asn+Asp and Gln+Glu were added compared with the addition of only Asp and Glu, suggesting that Asn, Gln or both were growth-limiting. This study did not consider Asn and Gln singly. Nonetheless, blood plasma AA concentrations in that study indicated that Asn may have been more relevant than Gln, with the limitation that the pool of individual AA in the blood plasma may have been affected by altered influx and efflux caused by other metabolic processes than the considered ones. No treatment effects on Asp, Glu and Gln were determined, irrespective of whether Asn and Gln were substituted. However, when no Asn and Gln were substituted, blood plasma Asn declined, apart from the same substitution level at which a decline in growth performance was observed.

Indications of glutamine being the next-limiting nonessential amino acid

A review by Selle *et al.*⁽³⁹⁾ compiled results of twenty publications that described the impacts of Gln supplementation on growth performance. Gln supplementation increased growth performance in most experiments, suggesting that Gln was a growth-limiting factor in the unsupplemented treatments. Two of the studies found that Gln supplementation decreased performance above a certain supplementation level, and one study stated that performance decreased below a certain supplementation level. Two studies each found that Gln supplementation generally decreased performance or increased one of the performance traits (average daily weight gain or the gain:feed ratio) while the other performance trait was not compromised. The twenty compiled publications were heterogeneous in several regards. For instance, different age ranges were investigated, and the stated dietary CP ranged from 17.7–24.4%. Therefore, the supply of all AA relative to the requirement of the birds differed widely among studies. Housing and management conditions also varied. Furthermore, most of the compiled studies investigated effects of Gln supplementation while some supplemented Gln

and Glu in combination. None of the compiled studies investigated the supplementation of other nonessential AA. Taken together, the review by Selle *et al.*⁽³⁹⁾ provides evidence that Gln can represent a relevant AA but it is difficult to identify a clear pattern under which conditions Gln supplementation impacts growth performance.

Synopsis on Asn or Gln being the next-limiting nonessential amino acid

Currently, it is not possible to unequivocally rank the limiting relevance of Asn and Gln. The findings from different studies implicating either Asn or Gln being the next-limiting nonessential AA are not necessarily a contradiction. Asp, Asn, Glu and Gln are very closely related in metabolism and exhibit a high degree of interconvertibility⁽³⁷⁾. Hence, it is possible that supplementation of one of those AA can compensate for a deficiency of any of the others.

The possibility that the interconversion among Asp, Asn, Glu and Gln may make the relevant AA undetectable can be exemplified in the study of Ibrahim *et al.*⁽³⁸⁾. As mentioned earlier in 'indications of asparagine being the next-limiting nonessential amino acid', blood plasma Asn concentrations declined at a certain substitution of Asn+Asp with Asp, while blood plasma concentrations of Asp, Glu and Gln were unaffected. This does not necessarily mean that Asn is the relevant AA. These results can be interpreted alternatively as a prioritisation of constant Gln concentrations in the blood plasma achieved by the conversion of Asn to Gln. Such a phenomenon would result in the decline of Asn concentrations in the blood plasma. A varying supply with other AA can influence concentrations of Asp, Glu, Asn and Gln in the blood plasma because those AA can be formed metabolically from most of the other AA⁽¹⁵⁾. The study by Ibrahim *et al.*⁽³⁸⁾ is the only one that investigated varying dietary Asp, Asn, Glu and Gln concentrations without changing digestible concentrations of all proteinogenic AA, including concentrations of digestible Asp+Asn and Glu+Gln.

Indications of nonspecific amino-nitrogen being the limiting factor

Alternative to the interpretation that Asn, Gln or both are next-limiting nonessential AA in low protein diets adequate in Gly_{equi}, the results of the aforementioned studies may indicate an insufficient supply with nonspecific amino-N. One study⁽³⁶⁾ demonstrated that nonspecific amino-N was a relevant factor because supplementation of different nonessential AA increased growth performance compared with the unsupplemented diet. This suggests that the supplemented nonessential AA were metabolically converted to one or more limiting nonessential AA. However, no supplementation attained the growth that was found for a diet adequate in nonessential AA. It remains unknown whether a higher supplementation of one of the nonessential AA other than Gly_{equi} can overcome the deficiency in nonessential AA of the unsupplemented diet. An inadequate supply with nonspecific amino-N might also partly explain the bigger effects of Asn and Gln supplementation compared with supplementation of Asp and Glu mentioned before because Asn and Gln contain more amino-N than Asp and Glu.

Potential to increase nitrogen utilisation efficiency

Potential by meeting the requirements of nonessential amino acids

The determined NUE ranged from 50–65% in the first 3 weeks of age, with diets similar to current industry standards^(e.g. 33,40). Reducing the dietary CP concentration tended to increase NUE in published studies, with a high variation among studies. This variation probably can be explained by varying concentrations of single digestible AA, including nonessential AA, relative to what the animals can utilise for protein accretion. Higher NUE compared with the current standard is feasible by adjusting concentrations of essential AA and Gly_{equi} using free AA. Experiments determined NUE values of 70%⁽¹³⁾, 75%⁽⁴¹⁾, 78%⁽³⁶⁾ and 80%⁽¹⁸⁾ alongside high growth performance in 3-week-old broilers with diets containing 16.3–17.5% CP. We are not aware of studies investigating the potential to increase the experimentally determined NUE by meeting the requirements of nonessential AA in birds older than 21 d. Such investigations would provide significant insights because N intake and N excretion are higher in older than in younger animals. Of note, this review only mentions NUE determined on the basis of quantified N intake and N excretion. Another possibility is to determine NUE on the basis of body weight and the assumption of a constant N content in body weight. However, a literature evaluation showed that the interquartile range of the N content in body weight was 27.5–35.1 g/kg in studies on broiler chickens published between 2016 and 2024⁽⁴²⁾.

A further increase in NUE above 80% without affected growth performance by meeting the requirements of further nonessential AA in addition to Gly and Ser seems possible if diets do not include ingredients low in AA digestibility, but an upper limit of NUE cannot be derived at present. By definition, the NUE is limited by N excretions via N contained in the urine and faeces. The N excreted via the urine mainly originates from degraded AA. Reasons for AA degradation include the supply with digestible AA above the possible protein accretion because the genetic potential to accrete protein is reached or because another AA limits protein accretion. Minimising these reasons for AA degradation is one strategy to increase NUE. AA required for maintenance are also degraded. The AA needed for maintenance cannot be estimated precisely at present because such an estimation requires knowledge on maintenance requirements for all proteinogenic AA, but maintenance requirements for nonessential AA will remain unknown until their relevance is recognised. However, the contribution of maintenance metabolism to incomplete N utilisation can be calculated on the basis of the maintenance requirement estimates of the Society of Nutrition Physiology⁽⁴³⁾ and the performance objectives of Aviagen⁽⁴⁴⁾. At a very low CP content in the diet of 15.5%, which is currently possible without affected growth performance in the age period of 7–21 d post-hatch (mean of 14.7% and 16.3% CP, see ‘limitations of nonessential amino acids in Gly_{equi}-adequate diets’), maintenance requirements for essential AA are estimated to limit the NUE by 2.1–3.2 percentage-units. Hence, maintenance requirements for all proteinogenic AA (essential and nonessential AA) may limit the NUE in this age period by roughly 4–6 percentage-units. Another factor limiting the NUE is the CP digestibility of the feed, which determines N excretion via the faeces. The amount of N excretion via the faeces cannot be generalised because the CP digestibility is highly variable between and within feed ingredients. For most commonly used feed ingredients, CP digestibility is between 85 and 90%⁽⁴⁵⁾. With a

CP digestibility limiting the NUE by 10–15% and the maintenance requirement of proteinogenic AA limiting the NUE by ~5%, there is little potential for increasing the NUE above 80–85%. In addition to AA digestibility and AA requirements for maintenance, NUE is limited by inevitable post-absorptive AA degradation. Estimating the post-absorptive AA degradation is difficult because influences include an energy supply from AA⁽⁴⁶⁾ and the availability of individual AA for protein biosynthesis. The availability of individual AA includes the AA influx from protein degradation in the course of protein turnover, which seems to depend on the type of proteolysis^(37,47). Mechanisms that probably lead to decreased AA degradation include lower muscle protein turnover in the state of low AA supply^(48,49). Another possible mechanism to increase NUE is urinary N being reabsorbed in the hindgut and used for the synthesis of nonessential AA^(50,51). This may lead to an increased NUE because protein accretion would be achieved with lower nonessential AA intake. The choice of feed ingredients in low CP diets can also lead to increased NUE. Diets very low in CP often are formulated with feed ingredients high in CP digestibility. In addition, low CP diets commonly include free AA and the digestibility of free AA is complete⁽⁵²⁾. Hence, lowering dietary CP usually results in a smaller limitation of the NUE by undigested CP. Taken together, a rough estimate is the potential to increase the NUE of broiler chickens to a level of 85% in the age period of 7–21 d post-hatch by meeting requirements of further nonessential AA considering the maintenance requirements for all AA and the CP digestibility of diets.

Potential by accepting submaximal growth performance

Increasing production performance by measures not related to AA nutrition, such as breeding, usually raises the NUE, thus leading to decreased N excretion per produced animal-based food. A decreased relevance of the maintenance requirement relative to the total AA requirement, which is in the order of 3–8% of the total requirement for individual essential AA based on maintenance requirement estimates of the Society of Nutrition Physiology⁽⁴³⁾ and the performance objectives of Aviagen⁽⁴⁴⁾, contributes to this⁽⁵³⁾. This principle is more differentiated when the influence on performance is caused by varying supply with a limiting AA. Increasing performance by increasing the supply with limiting AA can decrease the NUE because the additional performance per additional AA supply decreases, particularly when performance approaches the plateau. Conversely, studies have found an increasing AA utilisation efficiency when performance of animals was reduced as a consequence of essential AA deficiency (Fig. 2). This phenomenon has been described for methionine, lysine and tryptophan in broiler chickens^(54–56) and for eight AA in the rainbow trout^(57,58). This outcome may be explained by maximised AA utilisation efficiency (increment in AA accretion per increment in AA intake) at an AA intake level below what is needed for maximised AA accretion (Fig. 3). Potential physiological explanations for these results include the lower muscle protein turnover in the state of low AA supply^(48,49) and the reabsorption of urinary N in the hindgut that can be used for the synthesis of nonessential AA, as mentioned in ‘potential by meeting the requirements of nonessential amino acids’.

Increased NUE at an AA supply not allowing for maximum performance may be considered a conflict of targets. If the hypothesis of an increased AA utilisation efficiency at submaximal growth performance as the primary cause for increased NUE is valid, there is a range in intake of the limiting AA where the conflict

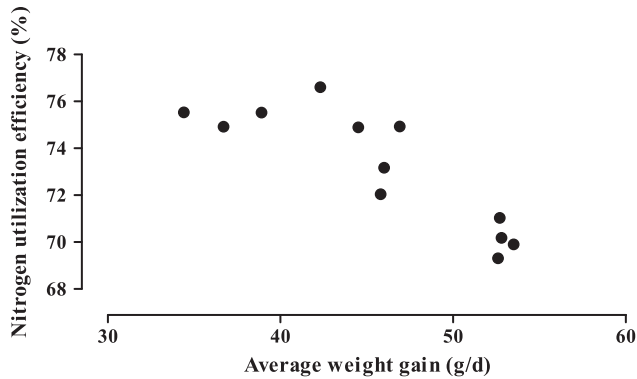


Fig. 2. Relationship between average daily weight gain and nitrogen utilisation efficiency⁽¹³⁾. Dots represent least square means ($n = 7$).

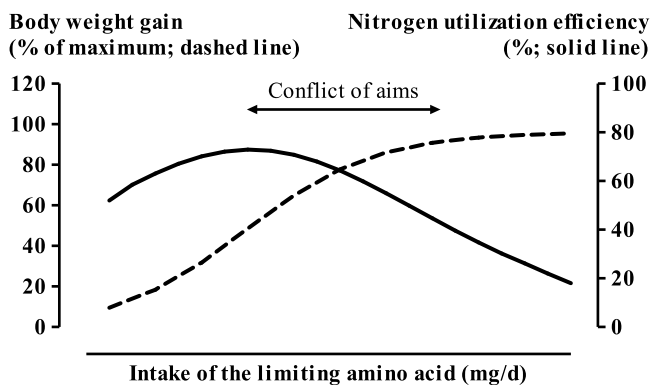


Fig. 3. Visualisation of the conflict of aims between maximising nitrogen utilisation efficiency and growth of broiler chickens depending on the intake of the limiting amino acid. Schematised responses are based on the body weight gain and the lysine utilisation efficiency determined previously.⁽⁵⁴⁾

of aims is relevant (Fig. 3). Lower intake of the limiting AA would lead to both impaired growth performance and NUE. At present, high growth performance is commonly considered the most important factor for diet formulation, and reduced nutrient excretion is of secondary priority. However, depending on the global availability of proteins and their costs, the framework may change to make production systems more profitable by accepting submaximal growth while increasing the utilisation of expensive and sparse protein sources. In some regions of the world (such as in some European countries), fertilizer legislation determines the animal production volume of a farm depending on the amount of N contained in the manure. Such constraints will likely become stricter and, hence, pose increasing relevance for the future.

Impacts of crude protein reduction on energy utilisation

Impacts on energy requirements

Many studies reported an increase in fat deposition in broiler chickens when dietary CP was reduced, although the diets were calculated to be isoenergetic and growth was not affected. This includes studies decreasing CP in Gly-supplemented diets^(24,32,59). Parts of this phenomenon may be explained by an affected energy provision from fermentation in the hindgut because the different ingredient composition of low CP diets can affect the substrates for the microbiota. Further, fat metabolism can be influenced by the

microbiome⁽⁶⁰⁾. In addition, parts of this phenomenon may be explained by an increased fat digestibility upon Gly supplementation in poultry^(61–64), with more Gly being available to form Gly-conjugated bile salts being a potential mechanism.

Another explanation for increased fat deposition when dietary CP is reduced is less energy being needed for N excretion because uric acid formation is energy-demanding. The energy needed for uric acid formation was calculated at 60.7 kJ/g of N excreted as uric acid⁽¹⁴⁾, which includes energy contained in uric acid and metabolic heat. A model calculation (Fig. 4) indicates that the energy needed for uric acid excretion decreases and fat accretion increases remarkably when dietary CP is reduced. This model calculation assumes that the metabolism will use the spared energy for fatty acid synthesis and fat deposition only. Proportions of uric acid in total urinary N tend to decrease with dietary CP^(13,17,36,65). Therefore, the energy requirement for uric acid synthesis probably becomes less pronounced relative to urinary total N excretion the more dietary CP is reduced. Spared energy in reduced-CP diets for N excretion reflects decreased energy requirements of the animals when the aim is not to increase fat deposition in the birds. Current dietary energy supply recommendations are based on studies using standard dietary CP concentrations. Therefore, avoiding increased fat deposition when using low CP concentrations in diets should ideally be done by concurrent adjustment of dietary energy.

Impacts on the nitrogen-corrected metabolisable energy

The N-corrected metabolisable energy (ME_N) is widely used as a reference unit for energy in poultry diets because the correction to zero N accretion results in energy concentrations that are barely dependent of energy excreted as urinary N⁽⁶⁶⁾. This makes determined ME_N concentrations in the feed largely unaffected of dietary CP and requirements for nitrogenous nutrients by the test animals. The correction to zero N accretion is performed by determining the energy that would have been needed if the accreted N had been excreted via urine. This energy is subtracted from the determined metabolisable energy (without correction to zero N accretion). The correction factor is 36.5 kJ/g N accretion and was determined in urine of birds fed diets containing ~23.5% CP⁽⁶⁷⁾. The urine in this study contained unknown amounts of uric acid, ammonia and urea, which are the compounds that contribute most to the energy in the urine of birds. Uric acid and urea contain more energy than ammonia⁽⁶⁸⁾. Hence, the energy in the urine of broiler chickens fed ~15% CP is most likely lower compared with the urine of birds fed higher dietary CP owing to a lower share of the urinary N from uric acid (see 'impacts on energy requirements') and the urea concentration is negligible⁽³⁶⁾.

A model calculation based on data from Hofmann *et al.*⁽¹³⁾ indicated that the impact of variations in urinary N composition upon feeding very low CP diets is insubstantial for dietary ME_N determination (Table 3). The estimated energy content of the urine according to varying uric acid and ammonia excretion differed between 30.2 and 33.5 kJ/g N, which is slightly lower than the energy in urinary N of 36.5 kJ/g reported in the aforementioned study that determined the energy in the urine of birds fed diets containing ~23.5% CP⁽⁶⁷⁾. Using individual factors to correct to zero N accretion caused the dietary ME_N concentrations to increase in the range of 0.06–0.09 MJ/kg compared with dietary ME_N determined with the constant correction factor of 36.5 kJ/g. In addition, the response pattern differences in dietary ME_N determined with constant and adjusted correction factors to the treatments were not statistically different.

Table 3. Model calculation on effects of constant or individual factors for correcting the metabolisable energy to zero nitrogen accretion based on previously published data⁽¹³⁾¹

		ME _N (MJ/kg) determined with constant factor to correct N accretion to zero ²	ME _N (MJ/kg) determined with individual factors to correct N accretion to zero ³
CP [%]	13.2	13.4 ^a	13.5 ^a
	14.7	13.3 ^b	13.4 ^b
	16.3	12.9 ^c	13.0 ^c
	Pooled SEM	0.019	0.022
Gly _{equi} [g/kg]	12	13.3 ^a	13.4 ^a
	15	13.2 ^{bc}	13.3 ^{bc}
	18	13.3 ^{ab}	13.3 ^{ab}
	21	13.2 ^c	13.2 ^c
	Pooled SEM	0.022	0.025

^{a-c}Values without a common letter differ significantly ($P \leq 0.05$) within the main effects CP and Gly_{equi}. Interactions between the main effects were not significant ($P > 0.05$)
¹CP, crude protein; Gly_{equi}, glycine equivalents; ME_N, nitrogen-corrected metabolisable energy; N, nitrogen; SEM, standard error of the means
²Dietary ME_N calculated according to the equation in ref. 66 with 36.5 kJ/g as the estimated energy concentration of urinary N and the factor to correct to zero N accretion according to ref. 67
³Dietary ME_N calculated according to the estimation equation in ref. 66 with values of 30.2–33.5 kJ/g as the estimated energy concentrations of urinary N and the factors to correct to zero N accretion assuming that urinary N only consisted of uric acid-N and ammonia-N. The proportions of measured uric acid-N and ammonia-N excretion relative to their sum were multiplied by their respective energy concentrations and then summed up to estimate the energy concentration of urinary N and to determine the individual factor to correct to zero N retention for each observation. The energy concentrations used were 34.5 kJ/g for uric acid-N and 24.8 kJ/g for ammonia-N⁽⁶⁸⁾

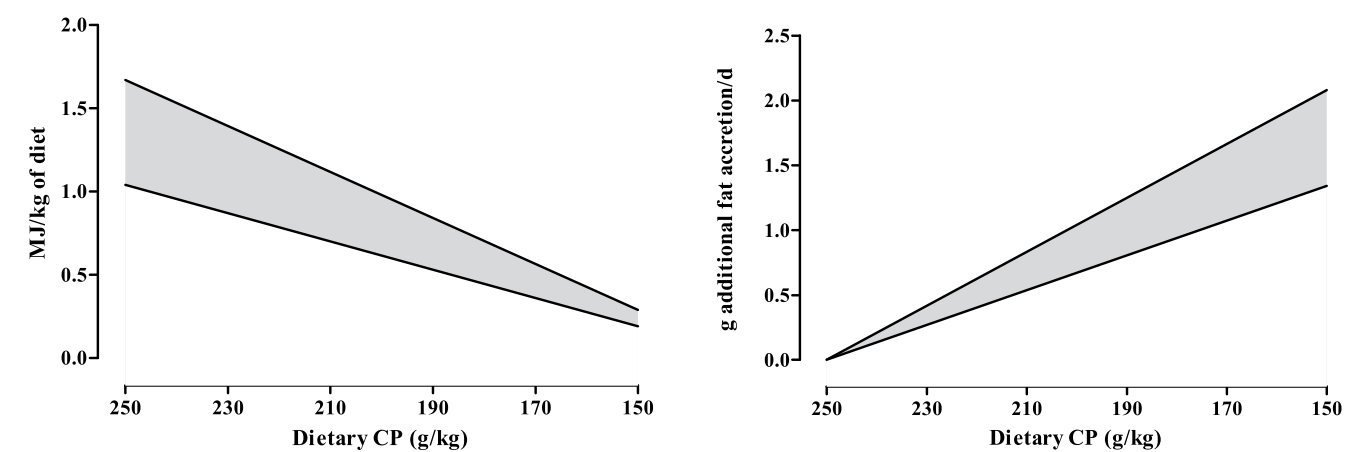


Fig. 4. Model calculation on the effect of dietary crude protein on energy requirement for uric acid synthesis (left panel) and corresponding fat accretion (right panel) in broiler chickens. Assumptions made are as follows: 90% prececal crude protein digestibility, 170 g protein/kg body weight gain⁽⁵⁶⁾, 59 g daily body weight gain, 75 g daily feed intake (performance objectives for 8–21 d of age⁽⁷⁰⁾), 60.7 kJ/g N excreted as uric acid including heat production⁽¹⁴⁾, 39.8 kJ/g energy accretion in body fat⁽⁴³⁾. The variable proportion of uric acid in total urinary nitrogen excretion (0.55–0.85⁽⁷¹⁾) is indicated by the grey area

Conclusions

An adequate supply of essential AA and Gly_{equi} enables the reduction of dietary CP in diets for broiler chickens to ~16% without compromising growth performance. Gly_{equi} requirements are variable, which probably is mainly due to varying uric acid production. Other influences discussed in the literature seem to exert their impacts on Gly_{equi} requirements predominantly by affecting uric acid production. A deficient supply with Asn, Gln or nonspecific amino-N is the most likely growth-limiting factor when dietary CP is reduced below 16%. Reducing dietary CP reduces energy requirements of the birds for N excretion. Lower energy expenditure for uric acid formation causes an increased fat deposition when dietary ME_N is not adjusted. Impacts of CP reduction on the composition of the urine have a negligible impact on the accuracy of ME_N values of feeds.

Data availability statement. No data availability statement is applicable for this review because all information is based on previously published data.

Authorship. Wolfgang Siegert: conceptualisation, formal analysis, methodology, resources, supervision, visualisation and writing – review and editing. Adewunmi Omotoso: data curation and writing – original draft. Philipp Hofmann: data curation, formal analysis, methodology and writing – original draft. Markus Rodehutscord: conceptualisation, resources, supervision and writing – review and editing.

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