Relevance of glycine in crude protein-reduced broiler nutrition

Abstract
This article reviews the possibilities to reduce the crude protein concentration in feed for broiler chickens. Considering concentrations of all essential amino acids allows for a reduction of the crude protein concentration in the feed for 1 to 21 days-old broiler chickens to about 19%, without impaired growth. Considering nonessential amino acids only as their sum does not allow for a reduction any further. Instead, single nonessential amino acids need to be considered. The nonessential amino acids glycine and allow for a serine are growth-limiting in feed for broiler chickens containing less than 19% crude protein. Glycine and serine are best assessed together as glycine equivalents (Gly\textsubscript{eq}) due to the equal effect of these amino acids on the growth. Adequate concentrations of Gly\textsubscript{eq} allow to reduce the crude protein concentration in the feed for 1 to 21 days-old broiler chickens to about 16% without implications on the growth. The requirement for Gly\textsubscript{eq} depends on the concentrations of other nutrients in the feed, such as threonine, choline, and cysteine. A reduction of the crude protein concentration below 16% is expected to be possible in the future by optimizing the Gly\textsubscript{eq} concentration in the feed and the factors influencing the response to Gly\textsubscript{eq}.

Keywords
broiler chickens, low protein feed, glycine, nonessential amino acids

Introduction
The increasing demand for meat and other animal products and the concomitant 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 the affordability of food in developing countries. Excreted nitrogenous compounds of broiler chickens and other farm animals can have negative effects on the environment, including soil, water and atmosphere. 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. Nitrogen excretion of animals can be reduced effectively by lowering the crude protein concentration in the feed. Experiments, however, showed that feed containing a crude protein concentration of less than 19 to 20% often had undesirable effects on the performance of 1 to 21 days-old broiler chickens even though the requirement of all essential amino acids was met.

About ten years ago, several potential reasons for unfavorable effects of low crude protein feed for broiler chickens have been discussed (Aftab et al., 2006). One of those reasons is a potentially different optimal concentration of essential amino acids between standard and low crude protein feed. This aspect was targeted in numerous studies. Some studies supplemented free amino acids to low crude protein feed to raise the level of all essential amino acids above recommendations (e.g. Jiang et al., 2005). This consistently did not overcome the reduced growth induced by low crude protein feed. In other studies, the concentrations of single essential amino acids or combinations of essential amino acid were varied (e.g. Si et al., 2004). This had no effect on the growth in most cases. Few studies reported an increased growth compared to the low crude protein feed. However, the growth of standard crude protein feed has not been met in any of the studies.

The effect of nonspecific nonessential amino-nitrogen was evaluated by investigating the effects of different combinations or levels of nonessential amino acids in the feed (e.g. Namroud et al., 2010). This had no effect on the growth in some studies. In other studies, a growth-increasing effect compared to the low crude protein feed was reported, but the level of growth of standard crude protein feed has not been achieved. Considering the sum of nonessential amino acids probably is not sufficient because specific metabolic processes can become limiting. This leads to the implication that single nonessential amino acids are important to avoid unfavorable effects of low crude protein feed on the growth of broiler chickens.

**Glycine in nutrition of broiler chickens**

Single nonessential amino acids have been supplemented to low crude protein feed in several studies. Supplementing free glutamic acid, aspartic acid, proline, and alanine consistently did not prevent from reduced growth caused by feeding low crude protein feed (Corzo et al., 2005; Dean et al., 2006). However, growth-increasing effects were determined when free glycine was supplemented. Two studies showed that supplementing feed with a crude protein concentration of 16% with free glycine to the level of about 22% crude protein control feed prevented reduction of growth compared to the control feed (Dean et al., 2006; Awad et al., 2015).

However, the concentration of glycine in nutrition of broiler chickens cannot be considered alone because studies revealed that serine in the feed has the same effect on the growth as glycine (Sugahara and Kandatsu, 1976). Animals can convert glycine into serine and vice versa. This interconversion seems to be not limited in poultry. The analogue effect of glycine and serine often is taken into account by calculating the sum of the concentrations of glycine and serine (Gly+Ser). However, this neglects the fact that glycine and serine in the feed are equally effective only when the same molar amount of those amino acids is considered. Therefore, Dean et al. (2006) proposed calculating the glycine equivalent (Glyequi) as the sum of glycine and the molar glycine equivalent of serine, calculated as follows:

\[
\text{Glyequi (g/kg)} = \text{glycine (g/kg)} + [0.7143 \times \text{serine (g/kg)}]
\]

where 0.7143 is the ratio of the molar weight between glycine and serine. Formulating feed using Glyequi is more appropriate than using Gly+Ser and its calculation is easy. Therefore, using Glyequi is recommended for future industry applications. Once calculated for the feedstuffs, Glyequi can be handled like any other single amino acid in feed formulation.

Glycine and serine are incorporated in almost all body proteins. Collagen and elastin are amongst the proteins richest in glycine. Keratin, which is mainly present in feathers and claws, contains high proportions of both glycine and serine. This might explain why a deficiency of glycine and serine in feed has been described to cause low skin strength (Christensen et al., 1994) and impaired feather development (Robel, 1977). Thus, supplying broiler chickens deficiently with Glyequi may cause economic implications in addition to the consequences of the low growth.

Meanwhile, it is widely accepted that Glyequi is the first-limiting nonessential amino acid in feed for broiler chickens (Ospina-Rojas et al., 2012). Waguespack et al. (2009) considered Glyequi to be the fourth-limiting of all proteinogenic amino acids after methionine, lysine, and threonine in feed based on corn and soybean meal for broiler chickens from 1 to 18 days. This was basically confirmed by Ospina-Rojas et al. (2014), who described valine and Glyequi as equally limiting after methionine+cysteine, lysine and threonine in corn-soybean meal-based feed for broiler chickens from 1 to 21 days.
Growth response to Gly$_{equi}$

Several dose-response studies investigating the effect of Gly$_{equi}$ in feed were published. A compilation of some studies shows that the growth response to incremental dietary Gly$_{equi}$ was inconsistent (Figure 1). This indicates that the response to Gly$_{equi}$ is influenced by other dietary factors.

The metabolic formation of cysteine from methionine dissipates Gly$_{equi}$ (Figure 2a) (Powell et al., 2011). This is especially important in low crude protein feed because a targeted methionine+cysteine concentration usually is achieved by adding DL-methionine or analogue supplements, whereas free cysteine usually is not added. The meta-analysis by Siegert et al. (2015a) showed that the cysteine concentration in the feed has a substantial impact on the gain to feed (G:F) response to Gly$_{equi}$ even though the methionine+cysteine concentration did not vary considerably (Figure 3). Fulfilling the requirement of broiler chickens for both methionine and cysteine reduces the necessity to convert methionine to cysteine and, therefore, reduces the requirement for Gly$_{equi}$.

Glycine and serine are nonessential amino acids and, therefore, can be produced to some extent by the metabolism of the birds. Several substances can be metabolized to glycine or serine out of which threonine and choline are the quantitatively most important (Meléndez-Hevia et al., 2009). Threonine can be metabolized directly to glycine (Figure 2b), and choline can be metabolized to glycine with betaine and dimethylglycine as intermediate steps (Figure 2c). The study of Siegert et al. (2015b) showed that the response to Gly$_{equi}$ depended considerably on the threonine concentration in the feed (Figure 4). An increase in the threonine concentration reduced the Gly$_{equi}$ concentration required to achieve certain response levels. The same study also revealed that the choline concentration influenced the response to Gly$_{equi}$ and threonine. Therefore, concentrations of Gly$_{equi}$, threonine, and choline need to be considered together in feed formulation. The observed potential of reducing the Gly$_{equi}$ concentration in feed by increasing the threonine and choline concentrations exceeded the theoretically possible replacement that can be explained by endogenous conversion processes. In consequence, the results shown in Figure 4 do not allow for the conclusion that adding threonine above recommendations prevents comprehensively from undesirable effects initiated by Gly$_{equi}$ deficiency. Reasons for that are currently unknown and should be investigated before recommendations are made that can be applied by feed formulators.
There are further possible but scarcely investigated influencing factors on the response to dietary Gly\textsubscript{equi}. One of them is arginine which, together with glycine, is a precursor of guanidino acetic acid. Guanidino acetic acid then reacts to form creatine (Figure 2d), a compound that is involved in the energy supply of animal cells including the muscles. Interaction effects for guanidino acetic acid and arginine on the growth were reported for broiler chickens in several studies (e.g. Dilger et al., 2013). However, little is known about the effect of Gly\textsubscript{equi} on the growth response to arginine and guanidine acetic acid in the feed.

**Gly\textsubscript{equi} concentration in feedstuffs**

The concentration of Gly\textsubscript{equi} varies considerably between and within types of feedstuffs (Table 1). However, the variation in the Gly\textsubscript{equi} concentration of the protein is low. It ranges in most cereals, cereal byproducts, brewery byproducts, oil seeds, and pulses between 7.3 and 8.3 g/100 g crude protein. Compared to vegetable feedstuffs, the variation of the ratio of Gly\textsubscript{equi} to crude protein is higher in animal byproducts. While it is low in fish meal, the ratio is very high in meat meal and particularly in meat and bone meal.

Because the Gly\textsubscript{equi} concentration in vegetable proteins is low, using only vegetable ingredients in feed formulation restricts the possibility to increase or decrease the ratio of Gly\textsubscript{equi} to crude protein of plant-based feed. In consequence, formulating feed with Gly\textsubscript{equi} concentrations that avoid undesirable effects on the growth using plant-based feedstuffs only leads to an excessive supply of the animals with other amino acids. This can be avoided by feeding animal-derived proteins to poultry but those are prohibited in some countries. Feed additives suitable for elevating the Gly\textsubscript{equi} concentration in feed are free glycine and L-serine, which are not approved in some countries at this time.

In countries where animal-derived proteins are prohibited and free glycine and L-serine are not approved, an adequate Gly\textsubscript{equi} supply can only be achieved by a surplus supply with crude protein. Environmental and possible downsides need to be accepted if this strategy is pursued. A deficient Gly\textsubscript{equi} supply in low crude protein feed might be reduced by a surplus supply of endogenous precursors like threonine and choline. Betaine and dimethylglycine as intermediate steps when glycine is formed from choline might also be suitable endogenous precursors. However, these

**Figure 3: Response of G:F to different dietary concentrations of Gly\textsubscript{equi} dependent on the level of Cys determined in a meta-analysis using 10 published studies that investigated the response of 1 to 21 days old broiler chickens (Siegert et al., 2015a).**

**Figure 4: Gly\textsubscript{equi} concentration in feed necessary to achieve 95% of maximum G:F responses of 7 to 21 days old broiler chickens depending on the threonine concentration in feed (modified from Siegert et al., 2015b).**
possibilities need to better investigated before the relevance of endogenous pre-
cursors of Gly equi in Gly equi-deficient feed can be better evaluated.

Current status and perspectives of crude protein reduction

During the past decades the crude protein concentrations have been lowered
basically because of knowledge of the re-
quirements for essential amino acids. This
enabled diminishing safety margins of
 crude protein supply but still ensured an
adequate supply of essential amino acids.

Current research continues to search for
ways to reduce the crude protein con-
centration in feed without compromising
growth (e.g. Corzo et al., 2005; Dean et al.,
2006). Currently common crude protein
concentrations in feed for broiler chickens
from 1 to 21 days are about 21 to 22% (Fi-
gure 5). Dean et al. (2006) summarized that
the growth of broiler chickens fed with
feed containing less than 19 to 20% crude
protein was reduced even when the requi-
rement for essential amino acids was met.

Considering additionally Gly equi enables to
reduce the crude protein concentration in
the feed considerably without undesirab-
le effects on the growth. Several studies
showed that the growth of broiler chi-
ckens from 1 to 21 days fed with feed con-
taining less than 19 to 20% crude protein
was reduced even when the requi-
renent for essential amino acids was met.

Factors influencing the response to Gly equi
such as those mentioned before were sub-
optimal in most published dose-response
studies with Gly equi. Optimizing both the
Gly equi concentration in the feed and the
factors influencing the response to Gly equi
should enable to reduce the crude protein
concentration in the feed even further. The

Table 1: Concentrations of Gly equi and ratio of Gly equi in crude protein of selected feedstuffs relevant for animal feeding based on nutrient concentrations extracted from AMINOdat® 5.0 (2016).

<table>
<thead>
<tr>
<th>Feedstuffs</th>
<th>Gly equi (g/kg dry matter)</th>
<th>Gly equi (g/100g crude protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter wheat</td>
<td>8.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Durum</td>
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<td>7.0</td>
</tr>
<tr>
<td>Corn</td>
<td>6.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Triticale</td>
<td>9.5</td>
<td>7.3</td>
</tr>
<tr>
<td>Oats</td>
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<td>7.3</td>
</tr>
<tr>
<td>Oil seeds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapeseed meal</td>
<td>31.7</td>
<td>7.9</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>41.6</td>
<td>7.8</td>
</tr>
<tr>
<td>Sunflower expeller</td>
<td>44.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Pulses</td>
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<td></td>
</tr>
<tr>
<td>Field beans</td>
<td>21.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Field peas</td>
<td>18.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Lupins</td>
<td>30.4</td>
<td>7.4</td>
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<tr>
<td>Non-animal byproducts</td>
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<td></td>
</tr>
<tr>
<td>Corn gluten feed</td>
<td>17.1</td>
<td>7.5</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>11.4</td>
<td>8.2</td>
</tr>
<tr>
<td>Distillers dried grains with solubles (wheat)</td>
<td>22.5</td>
<td>7.2</td>
</tr>
<tr>
<td>Brewer’s dried yeast</td>
<td>35.3</td>
<td>7.8</td>
</tr>
<tr>
<td>Animal byproducts</td>
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<td></td>
</tr>
<tr>
<td>Fish meal</td>
<td>64.3</td>
<td>6.4</td>
</tr>
<tr>
<td>Feather meal</td>
<td>130.1</td>
<td>14.7</td>
</tr>
<tr>
<td>Meat meal</td>
<td>11.4</td>
<td>14.8</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>14.9</td>
<td>17.7</td>
</tr>
</tbody>
</table>

Figure 5: Currently common crude protein concentration in feed for 1 to 21 days old broiler chi-
ckens and concentrations to which level the crude protein in feed can be reduced without affec-
ting growth by considering the concentrations of essential amino acids and Gly equi.
level to which the crude protein concentration in the feed with optimized concentrations of Gly_equi and of the factors influencing the response to Gly_equi has not been identified so far.

Glycine and serine are the first nonessential amino acids of which experimentally verified requirement values are available. To our knowledge, experimentally verified requirement values for other nonessential amino acids than glycine and serine are not available. It can be expected that the crude protein concentration in the feed can be reduced below 16% without adverse effects on the performance when the role of other nonessential amino acids needs is better understood and experimentally verified requirement values are available.

Conclusions
Gly_equi usually is growth-limiting when the crude protein concentration in feed for broiler chickens from 1 to 21 days is below 19%. Considering of the concentration of Gly_equi in feed enables to reduce the crude protein concentration in for broiler chickens to about 16%. The requirement for Gly_equi depends on the concentrations of other nutrients in feed like threonine, choline, and cysteine. It can be expected on the long term that the crude protein concentration in feed for broiler chickens can be reduced below 16% without adverse effects on the growth when the requirement of other nonessential amino acids is assessed.

References


