Glycine plus serine requirement of broilers fed low-protein diets

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Glycine plus serine requirement of broilers fed low-protein diets

A dose response study

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Samenvatting
In een dosis-respons studie met 910 Ross 308 vleeskuikenhanen, die werden gehuisvest in 70 grondhokken met strooisel, werd het effect van het glycine(+serine) niveau in laag-eiwitvoeders onderzocht in het leeftijdstraject van 10 – 35 dagen. In totaal werden vijf verteerbare glycine+serine niveaus, oplopend in evenredige stappen van 12,4 tot 15,7 g/kg in de groeifase en van 11,4 tot 14,9 g/kg in de eindfase, onderzocht. Daarnaast werd het mogelijk glycine-besparende effect van threonine bestudeerd; hiertoe werd aan het laag-eiwit voer met het laagste glycine+serine niveau extra threonine toegevoegd. De resultaten van de laag-eiwitvoeders werden vergeleken met een voer met een normaal eiwitgehalte met eenzelfde aminozurenprofiel.
Uit dit onderzoek kwam naar voren dat het glycine+serine niveau in laag-eiwitvoeders geen noemenswaardig effect had op de productieresultaten, slachtrendementen, strooiselkwaliteit en voetzoollaesiescore en dat een verteerbaar glycine+serine gehalte van 12,4 g/kg en 11,4 g/kg in respectievelijk het groei- en eindvoer voldoende is.

Summary
In a study with 910 Ross 308 male broilers, housed in 70 floor pens bedded with wood shavings, the effect of digestible glycine+serine content (5 levels ranging from 12.4 to 15.7 g/kg and 11.4 to 14.9 g/kg in grower and finisher diets, respectively) in low-protein diets was studied from 10 – 35 days of age. In this study, also the glycine-sparing effect of threonine was studied. In total seven different treatments were studied: a control treatment (a normal/high protein diet), five low-protein dietary treatments with increasing levels of digestible glycine+serine and a low-protein dietary treatment in which extra threonine was supplemented to the diet with the lowest glycine+serine level. Growth performance results, slaughter yields, litter quality, litter composition and footpad score were measured.
This study showed that the glycine+serine level in low-protein feed did not have a noticeable effect on the production results, slaughter yields, litter quality and foot pad lesion. Based on this study it was concluded that a digestible glycine+serine dose in low-protein diets of 12.4 g/kg and 11.4 g/kg in grower and finisher phase, respectively, is sufficient.

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Wageningen Livestock Research Report 1116

Table of contents

Foreword

Feed4Foodure is a public-private partnership between the Dutch Ministry of Agriculture, Nature and Food Quality, a consortium of various organizations within the animal production chain and Wageningen Livestock Research. Feed4Foodure aims to contribute to sustainable and healthy livestock farming in the Netherlands, simultaneously strengthening its competitive position on the global market. The Feed4Foodure program line “MVV5”, aims to reduce the dietary soybean meal content of Latin American origin, and thus reducing the amount of non-EU protein in the diet of pigs and poultry.
One of the options to reduce the amount of imported soybean meal content in the diet, is the use of low-protein diets, supplemented with free amino acids. The current report describes the results of a study in which the glycine+serine requirements and the glycine-sparing effect of threonine was determined in male broilers fed low-protein diets. For the current study, scientists of Wageningen Livestock Research worked together with representatives from the Feed4Foodure consortium. The authors thank the industry partners of the project team for their valuable input.

Marinus van Krimpen, project leader.

Summary

Providing diets to broilers with a reduced crude protein (CP) content that are supplemented with increased levels of free amino acids (AA) to cover birds AA requirements might be helpful in reducing the use of soybean meal of Latin American origin, and thus in reducing the amount of non-EU protein in broiler diets. For maintaining performance levels of broilers fed low-protein diets, the AA supply is essential. In literature, it is suggested that in low-protein diets glycine (Gly) and serine (Ser) may become growth limiting, but the optimal level of digestible Gly+Ser in low-protein diets is currently not clear. Moreover, it is known that threonine (Thr) is a precursor of Gly, and therefor Thr might have a Gly-sparing effect.
To clarify which level of digestible Gly+Ser is optimal under low-protein diet conditions, a dose-response study with male broilers was performed. Within this study, also the possible Gly-sparing effect of Thr was studied.

The study was carried out at the broiler trial facility of ForFarmers, Nijkerk, The Netherlands. This facility comprises 72 floor pens (0.75 m^2), of which 70 were used for this study. All pens were bedded with white wood shavings and 13 Ross 308 male broilers were housed in each pen. The lighting schedule was 23 h d^{-1} from 0 to 2d, and 18 h d^{-1} from 3 to 35d. The temperature was gradually decreased from 34 ºC (d0) to 20 ºC at 34d of age and this temperature was remained until the end of the of the experiment (35d of age).
Broilers received a 3-phase diet program: starter (0 to 10d), grower (10 to 28d) and finisher (28 to 35d of age). All broilers received the same standard starter diet, and subsequently seven different dietary treatments were applied: a control treatment (standard CP content), five low-protein dietary treatments with increasing concentrations of dGly+Ser (from 12.4 to 15.7 g/kg and from 11.4 to 14.9 g/kg, in grower (10- 28 d) and finisher phase (28 – 35 d) respectively), and a treatment in which 0.7 g/kg extra digestible threonine (dThr) was supplemented to the diet with the lowest dGly+Ser level.
The control diet program had a CP content in the grower and finisher phase of 209 and 199 g/kg, respectively. The CP content of the low-protein diets was 32 and 34 g/kg lower than the control in the grower and the finisher phase, respectively. All diets were formulated to meet or exceed the requirements concerning faecal digestible AA, and to be iso-caloric within each feeding phase. Feed and water were provided for ad libitum intake during the entire experimental period.

No differences in growth performance were observed between broilers fed the control diet program and those who consumed the low-protein diet programs. Feeding low-protein diets resulted in a better litter quality, better footpad health and a more efficient protein conversion, so less N is excreted. Digestible Gly+Ser level did not affect performance parameters or footpad score. Broilers fed the low-protein diet with the highest dGly+Ser level had the lowest breast meat yield, but it differed only significantly with the diet programs with the two lowest dGly+Ser levels. Extra dThr did not influence growth performance, litter score or footpad score, but it influenced breast meat yield negatively.

From this study, it can be concluded that dGly+Ser doses in low-protein diets of 12.4 g/kg and 11.4 g/kg in grower and finisher phase, respectively, are sufficient. It is possible to reduce the crude protein content of grower and finisher diet with 30 g/kg, provided that the amino acid balance and level in the diets meet the bird's requirements by supplementation of free (essential) amino acids. By this, the soybean meal inclusion level can be reduced by 46%, relatively.

1 Introduction

1.1 General

Reducing the crude protein (CP) content of poultry diets is an important way for the Dutch feed industry to reduce the import of soybean meal and the carbon footprint of animal production. Also, it is forecasted that protein sources will be scarcer in the future, due to the increased demand for human food. Consequently, costs of protein sources for feed may increase as well.

The CP content of broiler diets can, however, not be simply reduced, without considering the content of (semi-) essential amino acids (EAA). If these EAA become limiting, growth performance may be impaired, as protein/AA is required for gain. Therefore, it is essential to keep the supply and the balance of the most limiting amino acids in line with the requirement of the broilers, through proper feed formulation and an adequate dietary supplementation of free AA. Some studies showed that even if the (digestible) AA met the birds requirement, feeding low-protein diets resulted in reduced bird performance relative to birds fed standard diets (Dean et al., 2006; Namroud et al., 2008). This reduction in performance may be due to the underestimation of the glycine (Gly) plus serine (Ser) requirements in low-protein diets (Waterhouse and Scott, 1961; Dean et al., 2006). By reducing the dietary CP content, Gly+Ser levels decrease as well. Therefore, it is hypothesised that marginal levels of dietary Gly+Ser are the reason for the decrease in performance of broilers when dietary CP is decreased by more than 3%. Glycine supplementation was shown to completely recover bird's performance during the starter phase when these birds were fed a low-protein diet (Corzo et al., 2004; Dean et al., 2006; Ospina-Rojas et al., 2012). Van Harn et al. (2017) showed that dietary protein levels in the grower and finisher diets can be reduced up to 3% without deterioration of animal performance, provided that the supply of essential amino acids (including Gly), and their ratios relatively to lysine (Lys), are maintained on the level of the control diet.
In earlier studies within the framework of the Feed4Foodure project (Veldkamp et al., 2017), broiler performance (8-35 d) deteriorated after feeding a low-protein diet, despite most EAA (Lys, methionine, tryptophan, threonine, arginine, and isoleucine) were supplemented 10% above CVB (2012) recommendations, which was hypothesized to be the result of a lack of Gly and Ser.
Glycine is nowadays considered by some authors as an essential amino acid (Waguespack et al., 2009), although Ospina-Rojas (2013) found that a lack of Gly may especially limit performance in the starter phase.
Glycine content may become limiting, because of a lack of (or untimely) Gly precursor availability. Precursors of Gly are, among others, Ser, threonine, and choline (Siegert et al., 2015a). The amount of Gly available on gut level is influenced by availability of its precursors, but also by processes as the conversion of cysteine from methionine. The ratio methionine/cysteine may, consequently, affect the amount of Ser and of Gly that is available (Siegert et al., 2015b). Glycine is involved in the process of nitrogen excretion via the urine. Requirement for Gly may be increased beyond "normal" levels due to, e.g., an oversupply of protein, or of AA imbalance. These factors result in a higher production of nitrogenous waste products (ammonia) which need to be excreted, in poultry in the form of uric acid. The formation of the purine ring of uric acid requires, per molecule, one molecule of Gly.
The experiment was designed to get a better understanding of the influence of Gly in low-protein diets on performance. Basically, it concerns a Gly dose-response study in low-protein grower (10-28 d) and finisher (28-35 d) diets. The aim was to identify the ideal ratio digestible Gly (+ Ser) to digestible Lys, thus to extent the ideal protein matrix for broilers from 10 – 35 days of age. Within this experiment also the potential Gly-sparing effect of Thr was studied.

1.2 Glycine

Glycine is known as the simplest amino acid (NH_2CH_2COOH). The lack of a side chain of Gly results in physical characteristics like size, transmitting charge and hydrophobicity. Because of these features, Gly can settle in the hydrophobic interior of proteins which causes flexibility in the folding of proteins with a tendency to form helices. Moreover, Gly can adapt the structure of receptor sites and active sites of enzymes (Hall, 1998). Synthetic Gly is produced according to the Strecker method, by which amino acids are synthesized from ammonium chloride, hydrogen cyanide, and formaldehyde (Wendisch, 2007). It is used as a food additive due to its sweet taste and buffering capacity.
From all AA, Ser has the highest potential to be metabolized in Gly. When splitting the hydroxymethyl group of Ser, tetrahydrofolic acid (THF) receives the C1 unit and Gly is metabolized from Ser (Velišek and Cejpek, 2006). The enzyme serine hydroxyl-methyl-transferase (SHMT) functions as a catalyst in this reaction (see Figure 1.1 C). Although Ser is part of the Gly equivalent Gly+Ser, not all Ser is transformed into Gly. An effect of Cys exists on Ser, because Ser can also be used for the transformation of Met in Cys. If diets are supplemented with Met above requirements, Ser will be used to produce Cys. By this, the positive effects of Gly supplementation are reduced (Powell et al., 2011). When bird's requirements for Met and Cys are met, each molecule of Ser not needed for this conversion can be used for conversion into Gly, thus lowering the requirement of Gly (Siegert et al., 2015a).
Threonine is a known precursor of Gly via the Thr dehydrogenase and aldolase pathways (Davis and Austic, 1982), and it was earlier demonstrated that Thr could spare the Gly requirement (Ospina-Rojas et al., 2013; Siegert et al., 2015a).
Next to Ser, Thr can function as a precursor of Gly, because Thr is directly metabolized to Gly via the enzyme threonine aldolase (TA) with acetaldehyde as a by-product (Davis and Austic, 1982). This is also indirectly possible via threonine dehydrogenase (TDH) with 2-amino-3-ketobutyrate as an intermediate step, reacting further into Gly, acetyl-CoA, and amino acetone (Siegert et al., 2015a). These reactions are illustrated in Figure 1.1 A and B. From each molecule of Thr one molecule of Gly can be derived, but the replacement value due to endogenous conversion of one mass unit of Thr cannot exceed 0.63 mass unit of Gly (Siegert et al., 2015a). Ospina-Rojas et al. (2013) and Siegert et al. (2013) demonstrated that Thr could spare the Gly requirement.
Like Thr, choline can also be transformed into Gly if L-homocysteine is present following the steps choline- betaine aldehyde- betaine- dimethylglycine-sarcosine-glycine (Stekol, 1952). Choline levels of the broiler diet should be taken into account, as increasing choline intake reduces required Gly+Ser (Van Krimpen, 2016).

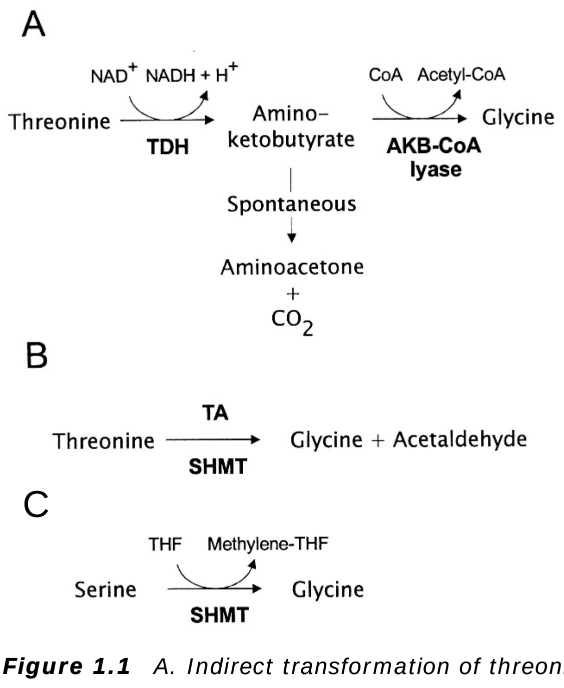

Figure 1.1 *A. Indirect transformation of threonine into glycine. B. Direct transformation of threonine into glycine. C. Direct transformation of serine into glycine. Adjusted from Simic et al. (2002).*

Glycine and Ser are, as any other AA, incorporated into proteins: collagen and elastin are proteins richest in Gly. In the primary structure, Gly is integrated at every third position. Keratin has both Ser and Gly in sufficient levels present. Keratin is utilised for feathers, beak and claws/nails in poultry. Proteins present in mucin are mainly Ser, because this provides binding sites for the oligosaccharide chains, which are of high proportion in mucins. Mucin is a substance creating a protection layer for the gut epithelium, which works against acidic conditions and proteases and functions as a selective diffusion barrier for nutrients. Furthermore, it fixates the commensal bacteria and serves as substrate for bacterial fermentation. Intestinal mucin secretion of broilers linearly increases with the level of Gly and Ser in diets (Ospina-Rojas et al., 2013). Glycine may also take part in regulation of some other mechanisms. For example, Gly plays a role in protein metabolism. When it is transferred in cells by Na^+ dependent transporters it regulates the cell protein metabolism (D'Mello, 2003). Glycine also contributes to the synthesis of haem and creatine. In the kidney, Gly converts Arg into guanidinoacetate, which becomes creatine in the liver. For the production of haem, Gly functions as the starting molecule. Glucogenic amino acids can be used in gluconeogenesis to produce glucose and adenosine triphosphate molecules (ATP). In this process, among others, Gly, Ser, and Thr result in pyruvic acid, which takes part in the citric acid cycle as pyruvate or indirect as Acetyl CoA to gain ATP (D'Mello, 2003).

Glycine and Ser are considered as non-essential amino acids (NEAA), because they can be synthesized by the broiler itself. However, under some conditions the synthesis could be insufficient for rapid growth (Leeson and Summers, 2001). Glycine is essential for the uric acid synthesis because it is needed for the formation of the purine ring in uric acid. Also DNA synthesis depends on purines made with Gly (Wang et al., 2013). Glycine is used in the formation of uric acid in which it converts 5-phosphoribosylamine in glycinamide ribonucleotide. The finalisation of uric acid synthesis is regulated by a molybdenum-containing enzyme named xanthine oxidase, which level changes with the protein level of the diet. The uric acid formed in the liver is excreted by the kidney with help of the tubules, which actively secrete the uric acid into the urine. The removal of uric acid from the blood is quite efficient. Chickens can excrete 5 grams of uric acid per day, which is necessary because uric acid is extremely insoluble. Otherwise blood levels may elevate resulting in uric acid under the skin and in the kidney, producing severe gout.
Since for every excreted uric acid molecule one molecule of Gly is lost, chickens have a high requirement for Gly (Leeson and Summers, 2001). Therefore it is argued that Gly is the fourth limiting amino acid, after Lys, Met and Thr (Waguespack et al., 2009). For example, in low-protein diets this limitation can be present because Gly and Ser levels decrease fast (Dean et al., 2006). Glycine and Ser can become growth limiting either by a lack of metabolic precursors for the various AA present, or because endogenous metabolism processes occur too slow (Berres et al., 2010). To reach maximal performance in broilers fed low-protein diets, supplemental free Gly may be essential (Ospina-Rojas et al., 2013). As different studies showed, the response to dietary Gly+Ser was inconsistent (Siegert et al., 2015b). Therefore, the aim of this study was to determine the optimal level of Gly+Ser in low-protein broiler diets. Also, the role of Thr as Gly-sparing AA in low-protein diets was investigated in the current project.

2 Materials and Methods

2.1 Study objective

The objective of this study was to evaluate the Gly+Ser requirements of male broilers fed low-protein diets from 10 – 35 days of age. Moreover, the Gly-sparing effect of Thr was studied.
Response parameters were: growth performance, slaughter yields, litter quality, litter composition and footpad lesions.

2.2 Start date and end date of the experiment

Start date: 23 May 2017
End date: 27 June 2017

2.3 Experimental animals

A total of 936 day-old male Ross 308 broiler chickens were used and allocated to 72 floor pens bedded with wood shavings (13 birds/pen). The broilers were sexed at the hatchery. The experiment started at 1 day of age and the experiment was completed at 35 days of age. All broilers were from the same parent stock and the age of the parent stock was 38 weeks.

2.4 Experimental design

The experiment was carried out at the experimental broiler house of feed producer ForFarmers, Nijkerk, The Netherlands. This natural ventilated house contained 72 floor pens of 0.75 m^2, of which 70 were used for this study (see Appendix 1). The remaining two pens were spare pens and used to standardize the number of animals in the experimental pens to 13 at the start of the experimental period (= day 10 of age). During the first ten days all broilers received a standard starter diet, and thereafter till 35 days experimental diets were provided. A complete randomized design with seven treatments was used in this experiment. Each treatment was replicated ten times and the experimental unit was a pen with 13 male broilers (Table 2.1). Treatments were completely randomly allotted among pens. The treatments and a description of the treatments are presented in Table 2.2.

Table 2.1 *Experimental design in summary.*

Item	Number
Total number of broilers	910
Total number of pens	70
Number of treatments	7
Replicates (pens) per treatment	10
Broilers per pen	13
Broilers per treatment	130

Table 2.2 *Experimental treatments.*

Treatment	Code	Description
A	12.4 GLY	Low-protein diet program [1] 12.4 / 11.4 g/kg dGly+Ser + 6.8 / 6.4 g/kg dThr (grower / finisher)
B	13.3 GLY	Low-protein diet program [1] 13.3 / 12.3 g/kg dGly+Ser + 6.8 / 6.4 g/kg dThr (grower / finisher)
C	14.1 GLY	Low-protein diet program [1] 14.1 / 13.2 g/kg dGly+Ser + 6.8 / 6.4 g/kg dThr (grower / finisher)
D	14.9 GLY	Low-protein diet program [1] 14.9 / 14.0 g/kg dGly+Ser + 6.8 / 6.4 g/kg dThr (grower / finisher)
E	15.7 GLY	Low-protein diet program [1] 15.7 / 14.9 g/kg dGly+Ser + 6.8 / 6.4 g/kg dThr (grower / finisher)
F	12.4 GLY+THR	Low-protein diet program [1] 12.4 / 11.4 g/kg dGly+Ser + 7.5 / 7.1 g/kg dThr (grower / finisher)
G	Control	High protein diet program [2] 15.7 / 14.9 g/kg dGly+Ser + 6.8 / 6.4 g/kg dThr (grower / finisher)

[1] CP level of 17.7% and 16.5% in in grower and finisher diet, respectively; [2] CP level of 20.9% in grower and 19.9% in finisher diet

2.5 Experimental diets

The experimental diets were formulated by ForFarmers and produced at the experimental feed plant of ForFarmers, Heijen, The Netherlands. A three-phase diet program was provided. Starter, grower and finisher diets were provided from 0 – 10, 10 - 28 and 28 – 35 days of age, respectively. During the starter phase all animals received the same diet with a crude protein content of 219 g/kg and 11.5 g/kg dLys. Thereafter, seven diet programs were provided: six low-protein and one control diet program. The crude protein content of the control diet program in the grower and finisher phase was 209 and 199 g/kg, respectively. The crude protein content of the low-protein diets was approx. 3% lower than the control diet in both the grower (177 vs. 209 g/kg) and finisher phase (165 vs. 199 g/kg). Digestible Lys content of the grower and finisher diets was 10.5 and 9.9 g/kg, respectively. Five low-protein diet programs (Treatment A – E) had graded amounts of dGly+Ser, ranging from 12.4 to 15.7 g/kg and 11.4 to 14.9 g/kg in grower and finisher phase, respectively. The sixth low-protein diet program (= Treatment F) was used to study the Gly-sparing effect of Thr. The diet composition of treatment F was similar with treatment A, but was supplemented with 0.7 g/kg extra dThr in both the grower and finisher phase. To create the different Gly(+Ser) steps (treatments B – E), free Gly was added 'on top' to diet A. The diets were formulated to meet or exceed the requirements concerning faecal digestible AA (lysine, methionine, threonine, tryptophan, isoleucine, valine and arginine) except for Gly and Ser, and to be iso-caloric within each period. All diets for each period were prepared with the same batches of ingredients. The ingredient and nutrient composition (incl. AA ratios relative to lysine) were established in consultation with the F4F project partners, and in-line with the previous low-protein study conducted within the framework of this project (Table 2.3; Van Harn et al., 2017).

The ingredient composition and the complete calculated nutrient contents of all diets are presented in Appendix 2 (grower diets) and Appendix 3 (finisher diets).
All diets were pelleted (starter diets as 2.5 mm diameter pellets, grower and finisher diets both as 3.2 mm diameter pellets) using a limited amount of steam (pelleting temperature < 70 ºC).

Table 2.3 *Calculated contents of energy (kcal/kg), crude protein and essential amino acids (g/kg) in the experimental diets.*

		Starter	Grower			Finisher		
			Control (G)	LP (A – E)	LP+Thr (F)	Control (G)	LP (A – E)	LP+Thr (F)
AMEn broiler	kcal/kg	2900	3000	3000	3000	3025	3025	3025
Crude protein	g/kg	219	209	177	177	199	165	165
Dig. Lysine	g/kg	11.5	10.5	10.5	10.5	9.9	9.9	9.9
Dig. Methionine	g/kg	5.5	5.0	5.4	5.4	4.7	5.5	5.5
Dig. Met+Cys	g/kg	8.5	7.9	7.9	7.9	7.5	7.5	7.5
Dig. Threonine	g/kg	7.5	6.8	6.8	7.5	6.4	6.4	7.1
Dig. Tryptophan	g/kg	2.4	2.3	2.3	2.3	2.2	2.2	2.2
Dig. Isoleucine	g/kg	7.8	7.4	7.4	7.4	7.0	7.0	7.0
Dig. Valine	g/kg	9.2	8.4	8.4	8.4	7.9	7.9	7.9
Dig. Arginine	g/kg	12.5	12.0	12.0	12.0	11.3	11.3	11.3
Dig. Gly + Ser	g/kg	16.5	15.7	12.4 – 15.7	12.4	14.9	11.4 – 14.9	11.4

2.6 Animal and housing conditions

A total number of 936 day-old male broilers Ross 308 were obtained from a commercial hatchery (Probroed & Sloot, Groenlo, The Netherlands), and equally distributed to 72 floor pens (floor space: 0.75 m^2) bedded with wood shavings (2 kg/m^2) in the natural ventilated poultry house of J.W. van Essen, Nijkerk, The Netherlands. Seventy of these 72 pens were used for this trial. In the remaining two pens, spare animals were housed, which were used to standardize the number of animals in the experimental pens in case of mortality during the starter phase (0 – 10d). During the first 10 days all animals received the same starter diet. At day 10 the number of animals were standardised to 13 per pen. After standardisation, the animals were weighed per pen and the experimental diets were provided.
The housing, management, feeding and husbandry conditions were standard and representative for a modern commercial broiler operation in Europe. Water and feed were provided for *ad libitum* intake during the entire experimental period (0 – 35 days of age). Feed was supplied via feeding bins (feeding space/pen 0.75 m). These bins were constructed in a way that feed spillage was limited. Water was supplied by one drinking cup (Impex) per pen (Figure 2.1). One day prior to placement of the broilers, the room was pre-heated to 30ºC. The temperature at the time of placement of the broilers was 34ºC and this temperature was gradually decreased to 20ºC at 34 days of age (Table 2.4). During the first four days the light was nearly continuous on (23L:1D), from 5 – 9 days of age a day/night schedule of 20h light and 4h dark (20L:4D), and from 9 – 35 days a day/night schedule of 18h light and 6h dark (18L:6D) was applied per 24h. Light intensity was 20 lux during the entire experimental period.
Visual observation of the birds was done twice per day to check animal health. All broilers were vaccinated against New Castle Disease (ND Clone 30, spray vaccination) at 15 days of age at the experimental facility.

Figure 2.1 *Left: experimental room; Right: experimental pen with 34 days old broilers.*

Table 2.4 *Temperature schedule.*

Age	Set temperature (°C)
0	34
2	33
4	32
6	31
8	30
10	29
14	28
18	27
21	25
28	22
34	20
34 – slaughter	20

2.7 Observations and measurements

- Diets

  After pelleting, and just before bagging, each diet was sampled (i.e. at intervals of 20 kg diet). These samples were pooled to form a composite, representative sample of each diet. All diets (1 starter, 7 grower and 7 finisher diets) were analysed on contents of dry matter, crude protein, crude fat, crude fibre, ash, starch and amino acids. All proximate analyses were performed at the laboratory of ForFarmers, Lochem, The Netherlands. The amino acid analyses were performed at Masterlab, Boxmeer, The Netherlands.

- Performance

  Body weight (BW) of birds per pen was determined at 0, 10, 28 and 35 days of age. Feed intake (FI) per pen was determined at 10, 28 and 35 days of age (provided feed minus remaining feed at 10, 28 and 35 days of age). Body weight gain (BWG), average daily gain (ADG), feed conversion ratio (FCR), feed intake (FI), and average daily feed intake (ADFI) were calculated on a pen basis from these data for the following periods: 0 – 10d, 10 – 28d, 28 – 35d, 10 – 35d and 0 – 35d. BWG = BW end period – BW start period; ADG = BWG / length period; FCR = (Total FI / (Total BW end period – total BW start period + total BW of dead or culled birds)); FI = FCR x BWG; ADFI = FI / length period. The crude protein conversion (CPC) was calculated over the above mentioned periods. CPC = FI (kg) x CP content diet (g/kg) / BWG (g). Culling, mortality and health were recorded daily (including probable causes of any culling, illness or deaths). EPEF (European Production Efficiency Factor) was calculated per pen at 35 days. EPEF was calculated as (mean daily body weight gain (g)/FCR*10) x (100 - % mortality).

  Twice daily, in the morning and the afternoon, animals and housing facilities were inspected by the farmer, thereby checking the general health status, availability of feed and water, as well as lighting, temperature and ventilation, and the occurrence of unexpected events. All incidences were recorded. Birds suffering pain or distress were selected, culled and the cause of the death or distress was recorded. In all cases of mortality, birds were weighed and date of death was recorded.

- Slaughter yields

  Slaughter yields of 10 randomly selected birds per pen were determined at 35 days of age. Selected birds were removed, individually marked and weighed and transported to a commercial slaughter house. At the slaughter house the broilers were cut up by hand by trained personnel to determine carcass, wing, leg (thigh + drums), back, breast meat and skin weight and yield. All yields were expressed as percentage of carcass weight, except carcass yield which is expressed as percentage of the live body weight. All measurements were performed by Plukon, Wezep, The Netherlands.

- Litter quality assessment
  Litter quality was visually scored at 10, 28 and 35 days of age by three persons, who scored independently the friability and wetness of the litter in each pen on a 1 (good) to 5 (bad) point scale. In Appendix 4 the scores and the description of each score are given.

- Litter composition
  At the end of the study representative samples of the litter were taken from all pens. These samples were analysed on: dry matter, total nitrogen (N), ammonium-N and pH. These analyses were conducted by the service lab of Wageningen Livestock Research, Wageningen, The Netherlands.

- Footpad lesions
  Occurrence of footpad lesions and their severity (scale 0, 1, 2) was determined at 34 days of age by an experienced assessor. All broilers per pen were used for these assessments. Footpad dermatitis was scored per broiler for both feet according to the so-called 'Swedish' classification, i.e. score 0: no lesions or very small discolouration; score 1: discoloration but no deep lesion; score 2: deep lesion with ulcers or scabs, bumble foot (Berg, 1998). An overview of scores for foot-pad lesions is presented in Appendix 5.
  The severity of footpad lesions was expressed as footpad score (FPS) per pen. This score is calculated as follows: 100% * ((0.5 * the total number of birds with score 1) + (2 * the total number of birds with score 2)) / the total number of scored birds. The flock FPS might range from 0 (all birds having no lesions) to 200 (all birds having score 2).

2.8 Statistical analysis

Raw data were analysed for statistical outliers. An outlier was defined as an observation which is deviating more than 2.5 times the standard deviations from the mean. If the deviation of an observation was more than 2.5 times the standard deviation from the mean and there was a plausible reason for this deviation, the observation was excluded from the dataset. The experimental data were analysed using GenStat statistical software (GenStat™ Release 18.1) on Windows 7.
The P-value of the treatment effect and the SEM (Standard Errors of Means) were provided per response parameter. Treatment effects with a P-value <0.05 were considered to be statistically significant.

Response parameters were analysed using ANOVA (analysis of variance) according the following model:

$$Y_{ij} = \mu + \text{Treatment}_i + \text{Error}_{ij}$$

Where:

Y	Response parameter
μ	General mean
Treatment	Effect of diet (i=1…7)
Error	Error term

Row and pen within row were included in the model as random terms.

3 Results

3.1 General

Average body weight of the chickens at arrival was 40 grams. Overall mortality during the experiment was 3,4%, which is slightly below the average Dutch standard (3.5%, KWIN 2017-2018). For this experiment, however, only male broilers were used and it is well known that male broilers have a higher mortality rate than female broilers. This means that the average mortality was low in this experiment.
Average body weight at 35 days was almost 30 g below (2254 vs. 2283 g), and average feed conversion ratio over the entire experimental period (0 – 35 days) was slightly lower (1.529 vs. 1.537 g/g) compared to the Ross 308 male broiler performance objectives (Aviagen, 2014) (Figure 3.1).

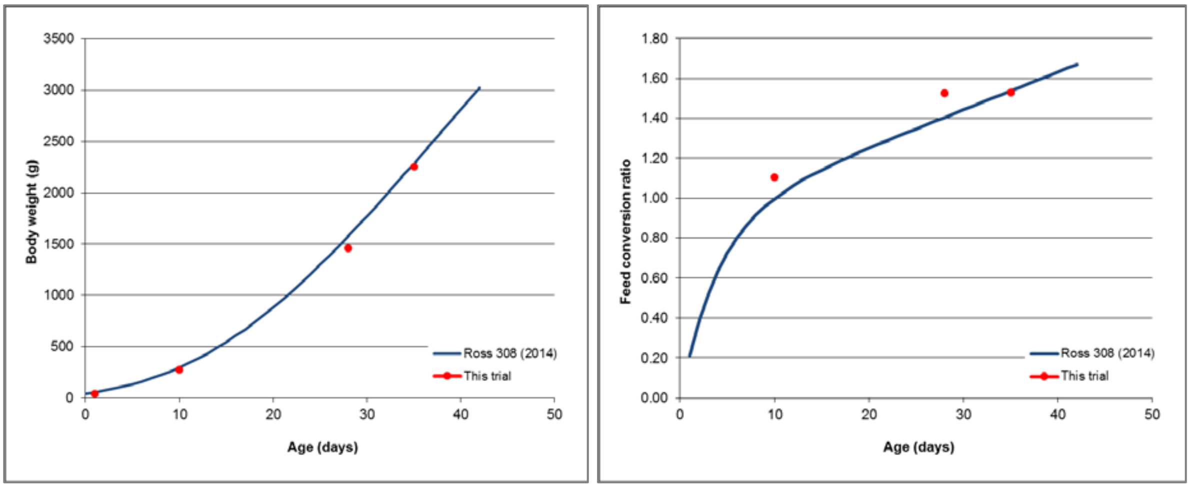

Figure 3.1 *Average body weight (left) and feed conversion ratio (right) observed in this experiment compared with the Ross 308 broiler male performance objectives (Aviagen, 2014).*

3.2 Diets

In Table 3.1, the calculated and analysed nutrient composition of the starter, grower and finisher diets are given.

From this table, it can be concluded that the analysed crude protein, crude fat, crude fibre, starch and crude ash content of all diets met the calculated contents, except for starch. On average, the analysed starch content was 2% lower than calculated.

In Table 3.2, the calculated and analysed amino acids (AA) contents of the starter, grower and finisher diets are given.

In general, the analysed AA contents met the calculated contents very well. In case of methionine (Met), cysteine (Cys), phenylalanine (Phe), tyrosine (Tyr) and proline (Pro), the differences between calculated and analysed contents were larger than 5 percent. The analysed Met and Cys contents were respectively 0.5 g/kg (= 9.0%) and 0.2 g/kg (= 5.5%) lower than calculated, whereas the analysed Phe, Tyr and Pro content were respectively 0.6 g/kg (= 7.3%), 0.7 g/kg (= 12.1%) and 0.8 g/kg (= 6.9%) higher than calculated.

Table 3.1 *The calculated and analysed nutrient composition (g/kg) of the starter diet, grower and finisher diets.*

		Starter	Grower diets							Finisher						
			12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control
Calculated																
Crude protein	g/kg	219	177	177	177	177	177	177	209	165	165	165	165	165	165	199
Crude fat	g/kg	65	61	61	61	61	61	61	78	57	57	57	57	57	57	75
Crude fibre	g/kg	35	34	34	34	34	34	34	36	34	34	34	34	34	34	36
crude ash	g/kg	54	44	44	44	44	44	44	49	39	39	39	39	39	39	43
Starch Brunt	g/kg	359	430	430	430	430	430	430	363	458	458	458	458	458	458	386
Analysed																
Crude protein	g/kg	220	177	181	181	180	182	177	205	166	165	168	169	168	166	197
Crude fat	g/kg	65	62	57	55	58	57	61	78	51	52	52	54	52	53	73
Crude fibre	g/kg	35	33	33	31	33	33	33	34	32	32	32	33	32	31	34
Crude ash	g/kg	52	44	44	43	45	43	44	48	38	38	39	38	37	38	43
Starch Brunt	g/kg	348	420	415	425	418	422	413	354	453	454	448	451	447	452	383

Table 3.2 *The calculated and analysed amino acids concentrations (in g/kg) of the starter diet, grower and finisher diets.*

	Starter	Grower							Finisher						
		12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control
Calculated															
Lysine (Lys)	12.9	11.5	11.5	11.5	11.5	11.5	11.5	11.8	10.8	10.8	10.8	10.8	10.8	10.8	11.2
Methionine (Met)	5.9	5.7	5.7	5.7	5.7	5.7	5.7	5.3	5.5	5.5	5.5	5.5	5.5	5.5	5.0
Cysteine (Cys)	3.7	3.1	3.1	3.1	3.1	3.1	3.1	3.6	2.9	2.9	2.9	2.9	2.9	2.9	3.5
Threonine (Thr)	8.8	7.9	7.9	7.9	7.9	7.9	**8.6**	8.1	7.4	7.4	7.4	7.4	7.4	**8.2**	7.7
Tryptophan (Trp)	2.7	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.4	2.4	2.4	2.4	2.4	2.4	2.5
Isoleucine (Ile)	8.9	8.2	8.2	8.2	8.2	8.2	8.2	8.5	7.7	7.7	7.7	7.7	7.7	7.7	8.0
Arginine (Arg)	14.0	13.0	13.0	13.0	13.0	13.0	13.0	13.4	12.2	12.2	12.2	12.2	12.2	12.2	12.6
Phenylalanine (Phe)	10.5	7.8	7.8	7.8	7.8	7.8	7.8	10.0	7.2	7.2	7.2	7.2	7.2	7.2	9.5
Histidine (His)	5.6	4.3	4.3	4.3	4.3	4.3	4.3	5.4	4.0	4.0	4.0	4.0	4.0	4.0	5.1
Leucine (Leu)	16.7	13.1	13.1	13.1	13.1	13.1	13.1	16.0	12.3	12.3	12.3	12.3	12.3	12.3	15.4
Tyrosine (Tyr)	7.5	5.6	5.6	5.6	5.6	5.6	5.6	7.1	5.2	5.2	5.2	5.2	5.2	5.2	6.8
Valine (Val)	10.6	9.5	9.5	9.5	9.5	9.5	9.5	9.8	9.0	9.0	9.0	9.0	9.0	9.0	9.3
Alanine (Ala)	9.6	7.7	7.7	7.7	7.7	7.7	7.7	9.3	7.2	7.2	7.2	7.2	7.2	7.2	8.9
Aspartic acid (Asp)	20.7	14.2	14.2	14.2	14.2	14.2	14.2	19.4	12.6	12.6	12.6	12.6	12.6	12.6	18.1
Glutamic acid (Glu)	42.5	33.3	33.3	33.3	33.3	33.3	33.3	40.9	31.0	31.0	31.0	31.0	31.0	31.0	39.1
Glycine (Gly)	9.0	**6.9**	**7.8**	**8.6**	**9.5**	**10.3**	**6.9**	**8.8**	**6.4**	**7.2**	**8.1**	**9.0**	**9.9**	**6.4**	**8.3**
Proline (Pro)	13.5	11.3	11.3	11.3	11.3	11.3	11.3	13.1	10.8	10.8	10.8	10.8	10.8	10.8	12.7
Serine (Ser)	10.4	7.8	7.8	7.8	7.8	7.8	7.8	10.0	7.2	7.2	7.2	7.2	7.2	7.2	9.5
Analysed															
Lysine (Lys)	12.7	11.4	11.4	11.4	11.2	11.2	11.3	11.9	10.7	10.5	10.6	10.7	10.6	10.8	11.3
Methionine (Met)	5.2	4.9	5.4	5.4	5.1	5.2	5.0	4.8	4.9	5.0	5.0	5.0	5.0	5.1	4.7
Cysteine (Cys)	3.5	2.9	3.0	2.9	2.8	2.9	2.8	3.3	2.7	2.8	2.7	2.6	2.8	2.8	3.3
Threonine (Thr)	9.0	7.8	8.0	7.7	7.6	7.8	**8.3**	8.3	7.5	8.0	7.5	7.6	7.5	**8.3**	8.0
Tryptophan (Trp)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoleucine (Ile)	9.3	8.6	9.0	8.4	9.1	9.1	8.6	9.6	7.7	7.6	7.6	7.5	7.3	7.6	8.3
Arginine (Arg)	14.4	13.2	13.5	13.0	13.3	13.2	12.9	13.6	12.5	12.2	12.5	12.5	12.3	12.5	13.1
Phenylalanine (Phe)	11.1	8.3	8.5	8.5	8.3	8.3	8.4	10.5	7.8	7.5	7.8	7.7	7.7	7.7	10.4
Histidine (His)	5.5	4.2	4.3	4.2	4.2	4.2	4.2	5.3	3.9	3.8	3.8	3.8	3.8	3.9	5.1
Leucine (Leu)	16.6	12.7	13.1	13.4	13.0	13.2	13.1	16.1	12.3	12.0	12.2	12.2	12.1	12.2	15.6
Tyrosine (Tyr)	8.2	6.4	6.4	6.5	6.3	6.2	6.2	7.9	5.8	5.7	5.9	5.9	5.8	5.8	7.6
Valine (Val)	10.3	9.2	9.3	8.8	9.4	9.7	9.4	10.1	9.7	9.4	9.4	9.2	9.0	9.0	9.5
Alanine (Ala)	9.6	7.4	7.5	7.6	7.5	7.4	7.5	9.0	7.2	7.0	7.1	7.1	7.0	7.1	8.9
Aspartic acid (Asp)	20.9	14.1	14.5	14.4	13.9	14.2	14.0	19.4	12.8	12.4	12.6	12.9	12.5	12.9	18.4
Glutamic acid (Glu)	43.9	33.7	34.2	34.4	33.9	34.1	33.6	41.8	32.0	30.9	31.2	32.1	31.8	31.7	40.3
Glycine (Gly)	9.2	**7.0**	**7.9**	**8.5**	**9.4**	**9.9**	**7.1**	**8.8**	**6.6**	**7.3**	**8.0**	**8.9**	**9.6**	**6.6**	**8.5**
Proline (Pro)	14.3	11.6	10.9	11.6	12.9	12.0	12.1	14.4	11.7	11.6	11.6	12.0	11.8	11.8	13.9
Serine (Ser)	10.5	7.7	7.9	7.8	7.7	7.7	7.7	9.8	7.3	7.1	7.2	7.4	7.3	7.4	9.7

3.3 Growth performance

This paragraph describes the growth performance results obtained in the grower phase (10 -28 days), finisher phase (28 – 35 days), grower and finisher phase (10 – 35 days) and the overall experimental period (0 – 35 days). In Appendix 6, the obtained growth performance results from 0 – 10 days (starter phase) and from 0 – 28 days (starter and grower phase) of age are given.

3.3.1 Grower phase

In Table 3.3, the growth performance results from 10 – 28 days of age are given. From this table it can be concluded that body weight (BW), body weight gain (BWG), mortality, feed conversion ratio (FCR) and feed intake were not affected by dietary treatment. Broilers fed the control diet had the highest crude protein intake and numerically the highest crude protein conversion. However, the crude protein conversion of the control diet did only differ significantly with the '14.1 GLY' low-protein diet. On average, the crude protein intake of the birds fed the low-protein diets was almost 40 gram lower compared with the CP intake of the broilers fed the control diet (343 g vs. 381 g). Since there were no differences in BWG, the crude protein conversion of birds fed the low-protein diets was on average 30 points lower than birds fed the control diet (0.291 vs. 0.321).
Performance results of male broilers fed low-protein diets were not affected by dGly+Ser level. Supplementing extra Thr to low-protein diets had no beneficial effect on the growth performance results of male broilers.

Table 3.3 *Growth performance results from 10 – 28 days (grower phase).*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Body weight (g)	1446	1434	1500	1497	1463	1424	1474	0.524	32.1
Body weight gain (g)	1172	1159	1227	1217	1189	1154	1196	0.581	31.4
Daily body weight gain (g/d)	65.1	64.4	68.2	67.6	66.0	64.1	66.5	0.581	1.74
Mortality (%)	3.8	1.5	0.8	3.8	1.5	1.5	1.7	0.458	1.25
Feed conversion ratio	1.656	1.646	1.568	1.583	1.605	1.663	1.567	0.523	0.0449
Feed intake (g)	1921	1899	1916	1920	1901	1908	1856	0.587	25.3
Feed intake (g/d)	106.7	105.5	106.5	106.7	105.6	106.0	103.1	0.587	1.41
Crude protein (CP) intake (g)	340.0 ^{b}	343.8 ^{b}	346.9 ^{b}	345.6 ^{b}	346.0 ^{b}	337.6 ^{b}	380.5 ^{a}	<.001	4.60
CP conversion (g/g)	0.293 ^{ab}	0.298 ^{ab}	0.284 ^{b}	0.285 ^{ab}	0.292 ^{ab}	0.294 ^{ab}	0.321 ^{a}	0.047	0.0083

^{a,b} Values without a common superscript per row differ significantly (P < 0.05).

3.3.2 Finisher phase

Like in the grower phase, during the finisher phase (28 – 35 days), no significant differences in growth performance results were found between the dietary treatments. On average the growth performance results of broilers fed low-protein diets did not differ from those who received the control diet. The dietary dGly+Ser level had no significant effect on any of the growth performance parameters. The inclusion of extra threonine had no beneficial effect on the growth performance results. As in the grower phase, the crude protein intake of broilers fed the low-protein diets was significantly lower compared with broilers fed the control diet (203 vs. 242 g). Birds fed the low-protein diets had also an improved crude protein conversion compared with broilers fed the control diet. The average CP conversion of all low-protein treatments was 0.260 g/g, whereas the CP conversion of the control diet was 0.322 g/g.

Table 3.4 *Growth performance results from 28 – 35 days (finisher phase).*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Body weight (g)	2263	2269	2254	2261	2260	2222	2238	0.814	23.8
Body weight gain (g)	811	836	754	763	797	797	764	0.633	35.1
Daily body weight gain (g/d)	115.9	119.4	107.7	109.1	113.9	113.9	109.1	0.633	5.01
Mortality (%)	0.8	0.0	0.0	0.8	0.8	0.8	2.6	0.249	0.74
Feed conversion ratio	1.548	1.507	1.626	1.581	1.545	1.543	1.633	0.718	0.0598
Feed intake (g)	1235	1240	1207	1191	1220	1207	1231	0.286	15.8
Feed intake (g/d)	176.4	177.2	172.5	170.1	174.3	172.5	175.8	0.286	2.25
Crude protein (CP) intake (g)	205.0 ^{b}	204.6 ^{b}	202.9 ^{b}	201.3 ^{b}	204.9 ^{b}	200.4 ^{b}	242.4 ^{a}	<.001	2.67
CP conversion (g/g)	0.257 ^{b}	0.249 ^{b}	0.273 ^{b}	0.267 ^{b}	0.259 ^{b}	0.256 ^{b}	0.322 ^{a}	<.001	0.0103

^{a,b} Values without a common superscript per row differ significantly ($P < 0.05$).

3.3.3 Grower and finisher phase

In Table 3.5, the growth performance results over the grower and finisher phase (10 -35d) are given.

On average the growth performance results of broilers fed low-protein diets did not differ from those who received the control diet program. Growth performance results of broilers fed the low-protein diets were not affected by the dietary Gly+Ser level. Also, the inclusion of extra threonine had no beneficial effect on the growth performance results. As the crude protein intake of the control diet was higher in both the grower and finisher phases compared with the low-protein diets, the crude protein intake over these combined periods was also higher (623 vs. 547 g). Birds fed the low-protein diets had an improved crude protein conversion compared with broilers fed the control diet. The average CP conversion of the low-protein treatments was 0.276 g/g, whereas the CP conversion of the control diet was 0.318 g/g.

Table 3.5 *Growth performance results from 10 – 35 days (grower + finisher phase).*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Body weight (g)	2263	2269	2254	2261	2260	2222	2238	0.814	23.8
Body weight gain (g)	1989	1994	1981	1980	1986	1952	1960	0.820	22.5
Daily body weight gain (g/d)	79.6	79.8	79.2	79.2	79.4	78.1	78.4	0.820	0.90
Mortality (%)	5.1	1.5	0.8	4.6	2.3	2.3	4.3	0.144	1.30
Feed conversion ratio	1.589	1.575	1.577	1.571	1.572	1.597	1.575	0.589	0.0111
Feed intake (g)	3157	3139	3124	3111	3121	3115	3087	0.880	35.4
Feed intake (g/d)	126.3	125.6	125.0	124.4	124.9	124.6	123.5	0.880	1.41
Crude protein (CP) intake (g)	545.3 ^{b}	548.4 ^{b}	549.7 ^{b}	546.9 ^{b}	551.0 ^{b}	538.1 ^{b}	623.0 ^{a}	<.001	6.27
CP conversion (g/g)	0.274 ^{b}	0.275 ^{b}	0.278 ^{b}	0.276 ^{b}	0.277 ^{b}	0.276 ^{b}	0.318 ^{a}	<.001	0.0020

^{a,b} Values without a common superscript per row differ significantly ($P < 0.05$).

3.3.4 Entire experimental period

In Table 3.6, the growth performance results over the entire experimental period (0 -35d) are given.

Over the entire experimental period none of the dietary treatments did alter BW, BWG, FCR or FI. Broilers fed the '12.4 GLY' diet program had the highest mortality rate. The mortality of this group, however, did only differ significantly from the broilers fed the '14.1 GLY' diet program. Performance results of male broilers fed low-protein diets were not affected by the dGly+Ser level. Supplementing extra Thr to low-protein diets low in dGly+Ser content did not have any beneficial effect on the growth performance results of male broilers. Broilers fed the control diet had the highest crude protein intake and the highest crude protein conversion. No differences were seen in crude protein intake and crude protein conversion between low-protein diets.

The footpad score (FPS) of broilers fed the control diet program tended to be higher than most of low-protein diet programs (P=0.057), which means that broilers of this group had the highest incidence and the most severe footpad lesions. No differences in FPS were observed between the low-protein diet programs.

Table 3.6 *Growth performance results from 0 – 35 days (entire experimental period).*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Body weight (g)	2263	2269	2254	2261	2260	2222	2238	0.814	23.8
Body weight gain (g)	2223	2229	2214	2221	2220	2182	2198	0.814	23.8
Daily body weight gain (g/d)	63.5	63.7	63.3	63.4	63.4	62.3	62.8	0.814	0.68
Mortality (%)	6.8 ^{a}	1.5 ^{ab}	0.8 ^{b}	4.6 ^{ab}	2.3 ^{ab}	3.1 ^{ab}	4.3 ^{ab}	0.039	1.34
Feed conversion ratio	1.533	1.526	1.530	1.520	1.522	1.544	1.525	0.718	0.0103
FCR 2300g	1.541	1.533	1.539	1.528	1.530	1.560	1.538	0.646	0.0126
Feed intake (g)	3407	3402	3387	3375	3379	3367	3352	0.948	36.9
Feed intake (g/d)	97.3	97.2	96.8	96.4	96.5	96.2	95.8	0.948	1.05
Crude protein (CP) intake (g)	600.3 ^{b}	606.1 ^{b}	607.6 ^{b}	605.0 ^{b}	607.8 ^{b}	593.4 ^{b}	681.4 ^{a}	<.001	6.63
CP conversion (g/g)	0.270 ^{b}	0.272 ^{b}	0.274 ^{b}	0.272 ^{b}	0.273 ^{b}	0.272 ^{b}	0.310 ^{a}	<.001	0.0019
EPEF [1]	386 ^{C}	411 ^{A}	410 ^{A}	398 ^{ABC}	407 ^{AB}	391 ^{BC}	394 ^{ABC}	0.085	7.0
FPS [2]	41 ^{B}	68 ^{AB}	62 ^{AB}	44 ^{B}	43 ^{B}	46 ^{B}	101 ^{A}	0.057	14.66

^{a,b} Values without a common superscript per row differ significantly ($P < 0.05$).

^{A,B,C} Values without a common superscript indicate a tendency ($0.05 \leq P < 0.10$).

[1] European Production Efficiency Factor; EPEF = (daily body weight gain (g)/FCR*10) x (100 - % mortality).

[2] Footpad score; FPS =100% * ((0.5 * the total number of birds with score 1) + (2 * the total number of birds with score 2)) / the total number of scored birds

The footpad scores (FPS) were inversely correlated with the dry matter content of the litter (see paragraph 3.5): dryer litter resulted in lower FPS (Figure 3.2).

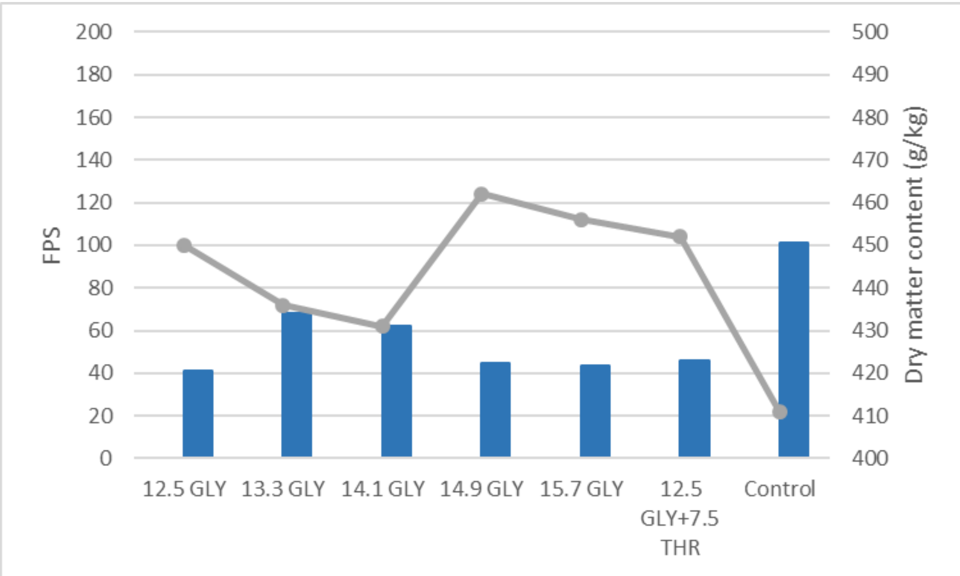

Figure 3.2 *Graphic representation of the litter dry matter content (grey line) and the footpad score (FPS – blue bars) per treatment.*

3.4 Slaughter results

In Table 3.7, the slaughter yields per treatment on 35 days of age are given. No significant effects on live weight, carcass weight and weight of different carcass parts were observed between treatments. There were, however, significant differences between dietary treatments in wing, leg and breast meat yield. Broilers fed the control diet program had a higher wing yield than broilers fed with one of the low-protein diet programs. Broilers fed the 15.7 GLY low-protein diet program had the highest leg (thigh + drum) yield, but the leg yield did only significantly differ from the control group, but not from the other low-protein diet treatments. Broilers fed the 15.7 GLY low-protein diet program had a significant lower breast meat yield compared with broilers who consumed the 12.4 GLY, 13.3 GLY and control diet programs. The breast meat yield of the 15.7 GLY group did not differ from the other low-protein diet programs.

Table 3.7 *Slaughter weight and yields of different carcass parts at 35 days of age.*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Live weight (g)	2284	2291	2277	2284	2270	2253	2270	0.938	23.1
Carcass weight (g)	1531	1541	1527	1535	1522	1518	1508	0.799	15.4
Carcass (%)	67.0	67.3	67.1	67.2	67.0	67.3	66.4	0.227	0.25
Wing (g)	159	160	160	160	159	158	161	0.769	1.5
Wing (%)	10.5 ^{b}	10.4 ^{b}	10.5 ^{b}	10.5 ^{b}	10.5 ^{b}	10.4 ^{b}	10.7 ^{a}	<.001	0.04
Leg (thigh + drums) (g)	525	530	527	528	530	524	515	0.446	5.3
Leg (%)	34.3 ^{ab}	34.4 ^{ab}	34.5 ^{ab}	34.4 ^{ab}	34.8 ^{a}	34.5 ^{ab}	34.1 ^{b}	0.015	0.13
Back (g)	245	246	245	247	245	245	242	0.947	2.7
Back (%)	16.0	16.0	16.0	16.1	16.1	16.2	16.1	0.817	0.08
Breast meat (g)	503	505	498	501	488	491	496	0.375	6.0
Breast meat (%)	32.8 ^{a}	32.8 ^{a}	32.6 ^{ab}	32.6 ^{ab}	32.0 ^{b}	32.3 ^{ab}	32.8 ^{a}	0.003	0.15
Skin (%)	3.2 ^{ab}	3.2 ^{ab}	3.2 ^{ab}	3.3 ^{a}	3.3 ^{a}	3.3 ^{a}	3.0 ^{b}	<.001	0.05

^{a,b} Values without a common superscript per row differ significantly ($P < 0.05$).

3.5 Litter quality and litter composition

In Table 3.8, the visual litter quality (friability and wetness) at 10, 28 and 35 day of age are given.

Table 3.8 *Visual litter quality on 10, 28 and 35 days of age.*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Friability D10 [1]	1.1	1.1	1.1	1.1	1.1	1.0	1.1	0.987	0.07
Wetness D10 [2]	1.3	1.3	1.3	1.2	1.3	1.1	1.3	0.808	0.10
Friability D28 [1]	3.2 ^{AB}	3.2 ^{AB}	3.2 ^{AB}	2.8 ^{B}	3.0 ^{AB}	3.2 ^{AB}	3.6 ^{A}	0.068	0.17
Wetness D28 [2]	2.6 ^{ab}	2.7 ^{ab}	2.6 ^{ab}	2.3 ^{b}	2.5 ^{ab}	2.5 ^{ab}	3.0 ^{a}	0.028	0.13
Friability D35 [1]	4.1 ^{abc}	4.5 ^{ab}	4.5 ^{ab}	3.8 ^{c}	3.9 ^{bc}	3.9 ^{bc}	4.7 ^{a}	<.001	0.15
Wetness D35 [2]	3.4 ^{ab}	3.5 ^{ab}	3.6 ^{ab}	2.8 ^{b}	3.0 ^{b}	3.0 ^{b}	3.9 ^{a}	<.001	0.19

^{a,b,c} Values without a common superscript per row differ significantly ($P < 0.05$).

^{A,B} Values without a common superscript indicate a tendency ($0.05 \le P < 0.10$).

[1] 1 – 5: 1 completely friable – 5 completely caked; [2] 1 - 5: 1 dry – 5 very wet.

The litter quality deteriorated as the broilers became older. In general, the litter quality of broilers fed the low-protein diet programs was better than the litter quality of broilers who received the control diet program. At 28 days, the litter of broilers fed the '14.9 GLY' diet was dryer (P=0.028) and tended to be more friable (P=0.068) compared with the litter of control group. At 28 days no differences were observed in visual litter quality between the low-protein treatments. At 35 days, the visual litter quality of the pens with broilers fed the ‘14.9 GLY’ or the ‘15.7 GLY’ low-protein diet program was significantly better than the control. The litter of these groups was more friable and less wet than the control group. The litter quality of the pens with broilers fed the low-protein diet programs with the lowest three dGly+Ser levels was numerically not statistically better than the control. Between the low-protein diet programs no differences were observed in wetness. The litter of broilers fed the 14.9 GLY low-protein diet program was more friable than the litter of broilers fed 13.3 GLY and 14.1 GLY low-protein diet programs at d35. The friability of the litter from this diet program did not differ from the other low-protein diet programs (12.4 GLY, 15.7 GLY and 12.4 GLY + 7.5 THR).

Litter characteristics are presented in Table 3.9. The results of the visual litter quality on day 35 agreed well with the dry matter (DM) contents of the litter on day 35. Broilers fed the control diet program had the lowest DM-content of the litter. The DM-content of the control group did differ from the 12.4 GLY, 14.9 GLY, 15.7 GLY and 12.4GLY+THR low-protein diet programs, but not from the other two low-protein diet programs.

The total-N content of the litter of broilers fed the 12.4 GLY, 13.3 GLY and 14.1 GLY low-protein diet programs was significantly lower than the litter of broilers fed the control diet program. The total-N content of the litter of broilers fed the other three low-protein diet programs was only numerically lower than the control. On dry matter basis the total-N content of the litter of all low-protein diet programs were significantly lower than the litter of broilers fed the control diet. On dry matter basis the ammonium N content of the litter of broilers fed the low-protein diets was lower than the litter ammonium N content of broilers fed the control diet. Numerically the pH of the litter of broilers fed the low-protein diets was higher than the pH of the litter of broilers fed the control diet, but only the 14.9 GLY, 15.7 GLY and 12.4 GLY+THR low-protein diet program differed significantly from the control diet program. No differences in litter pH were found between the low-protein diet programs.

Table 3.9 *Litter characteristics (DM, total-N, ammonia-N and pH) at 35 days of age.*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Dry matter (g/kg)	450.4 ^{a}	436.2 ^{ab}	431.4 ^{ab}	462.1 ^{a}	455.9 ^{a}	451.8 ^{a}	411.4 ^{b}	0.038	11.32
Total Nitrogen (g/kg)	16.11 ^{b}	16.06 ^{b}	15.47 ^{b}	16.94 ^{ab}	16.56 ^{ab}	16.43 ^{ab}	17.89 ^{a}	0.001	0.377
Total Nitrogen (g/kg DM)	35.92 ^{b}	36.82 ^{b}	35.92 ^{b}	36.84 ^{b}	36.32 ^{b}	36.41 ^{b}	43.51 ^{a}	<.001	0.619
Ammonium N (g/kg)	3.87 ^{b}	4.09 ^{ab}	3.92 ^{b}	4.20 ^{ab}	4.24 ^{ab}	4.22 ^{ab}	4.71 ^{a}	0.003	0.143
Ammonium N (g/kg DM)	8.79 ^{b}	9.40 ^{b}	9.12 ^{b}	9.20 ^{b}	9.33 ^{b}	9.37 ^{b}	11.44 ^{a}	<.001	0.389
pH	6.90 ^{abc}	6.91 ^{abc}	6.78 ^{bc}	7.12 ^{a}	7.07 ^{ab}	7.06 ^{ab}	6.66 ^{c}	0.037	0.11

^{a,b,c} Values without a common superscript per row differ significantly ($P < 0.05$).

4 Discussion

In the current trial, low-protein diets (17.7 and 16.5% CP in grower (10-28 d) and finisher (28-35 d) diet, respectively), supplemented with essential amino acids up to CVB recommendations (CVB, 2012), were fed to male broilers. The diets were supplemented with free Gly, to realise digestible Gly+Ser levels of 12.4, 13.3, 14.1, 14.9, and 15.7 g/kg in case of the grower diets and 11.4, 12.3, 13.2, 14.0 and 14.9 g/kg in case of the finisher diets, or with 0.7 g/kg extra threonine (as a precursor for Gly). The hypothesis was that these treatments allowed us to estimate the “optimal” dose of Gly in low-protein diets. Unexpectedly, the results of this trial showed that there was no response of free Gly on any of the growth performance parameters (body weight gain, feed intake or feed efficiency).

The results of this experiment were in contrast with those of Ospina-Rojas et al. (2013), who reported a linear increase of BWG and gain to feed ratio of broilers from 21 – 35 days of age when increasing dietary total Gly+Ser concentration in low-protein diets from 1.47 to 1.77%. Ospina-Rojas et al. (2013) also suggested that the requirement for total Gly+Ser concentration in this life phase may be beyond 1.77%, as the linear response of gain to feed ratio did not plateau. In the present study the total Gly+Ser content ranged from 1.48 to 1.81% in the grower diets (10 – 28d) and from 1.36 to 1.71% in the finisher diets (28 – 35d), which was in line with concentrations tested in the study of Ospina-Rojas.

Ospina-Rojas et al. (2013) also reported that the dietary requirement for Gly+Ser reduces when extra Thr was supplemented. In their study, supplementing 10% extra Thr (0.77% instead of 0.70% dThr) to low-protein diets resulted in a better feed efficiency. They concluded that Thr requirement in low-protein diets should be higher than the recommended value of 0.7% dThr. In the current experiment, no additional effects of Thr were observed. According to Ospina-Rojas et al. (2013) it is known that the enzymes Thr aldolase and Thr dehydrogenase catabolize excess Thr into Gly, thereby conserving the energy used in the synthesis of endogenous Gly (9 ATP/mol) and, thus, increasing the amount of energy available for optimal growth.

Table 4.1 *Differences in crude protein (g/kg) and amino acids composition (g/kg) between Ospina-Rojas et al. (2013) and the present study.*

	This experiment		Ospina–Rojas et al. (2013)
	10 – 28 days	28 – 35 days	21 – 35 days
Crude protein	177	165	168
Lys (Dig. Lys)	11.5 (10.5)	10.8 (9.9)	11.9 (10.8)
M+C	8.8	8.4	8.7
Thr (Dig. Thr)	7.9 (6.8)	7.4 (6.4)	8.1 (7.0)
Val	9.5	9.0	9.5
Arg	13.0	12.2	12.5
Ile	8.2	7.7	8.1
Gly+Ser	14.8	13.6	14.7

As mentioned earlier, some studies showed that Gly (+ Ser) in low-protein diets were limiting performance at young age (Corzo et al., 2004; Dean et al., 2006; Ospina-Rojas et al., 2012). This was, however, not confirmed in a study of Siegert et al. (2015a).

Veldkamp et al. (2017) hypothesised that the deteriorated growth performance of broilers in their low-protein experiments was probably caused by a lack of dietary Gly. The current findings, however, demonstrated that dietary Gly in low-protein diets is not growth limiting in the latter part of the growing period of broilers.

Kriseldi et al. (2017) conducted an experiment in which the effects of feeding low-protein diets supplemented with Gly and/or L-Gln (nitrogen source) on growth performance and carcass characteristics of broilers from 0 - 41 days was studied. From this study, it was concluded that Gly had a more pronounced effect than nitrogen contribution on FCR and BWG in starter and grower phase, whereas nitrogen contribution improved FCR in the finisher phase. In the study of Kriseldi et al. (2017), the positive effect on BWG occurred primarily in the grower phase (15 – 28 d), whereas the effect on FCR occurred both in the starter (0 – 14 d) and grower phase (15- 28 d). The study of Kriseldi et al. (2017) was carried out with diets based on corn and soy, whereas the diets in the present study were based on wheat, corn, soy and rapeseed meal. The crude protein level in Kriseldi's experiment was higher than in our experiment: 193 vs. 177 and 177 vs. 165 g/kg crude protein in grower and finisher phase, respectively. Also, the ratio between essential amino acids (EAA) and non-essential amino acids was different between both experiments: Kriseldi used a EAA:NEAA ratio of 55:45 in both grower and finisher phase, whereas the current study had a 48:52 ratio in the diets. It could well be that the difference in EAA:NEAA ratio was one of the reasons why no effects of additional Gly were observed, since feeding diets with an amino acid imbalance will lead to an increase of uric acid production/excretion, and thus to a higher Gly need.

Table 4.2 *Differences in crude protein (g/kg) and amino acids (g/kg) composition between Kriseldi et al. (2017) and present study.*

	Grower phase		Finisher phase	
	This experiment	Kriseldi et al.	This experiment	Kriseldi et al.
Crude protein	177	193	165	177
Dig. Lys	10.5	11.0	9.9	10.0
Dig. Met	5.4	5.9	5.5	5.4
Dig. M+C	7.9	8.4	7.5	7.7
Dig. Thr	6.8	7.6	6.4	6.9
Dig. Val	8.4	8.5	7.9	7.8
Dig. Arg	12.0	11.6	11.3	10.5
Dig. Trp	2.3	2.0	2.2	1.8
Dig. Ile	7.4	7.5	7.0	6.9
Total Gly	6.9	7.7	6.4	7.0
Total Gly+Ser	14.8	16.9	13.6	15.3
Total Gly equivalent [1]	12.5	14.3	11.5	12.9
Total EAA:NEAA [2]	48:52	55:45	48:52	55:45

[1] Total Gly equivalent (%) = total Gly (%) + [total Ser (%) × 0.7143], where 0.7143 is the ratio of the molar weight of Gly and Ser.

[2] Ratio of total essential to non-essential amino acid nitrogen was calculated by adding up the total nitrogen content in essential and non-essential amino acids. Essential amino acids are represented by Met, Lys, Thr, Val, Ile, Arg, Trp, His, Phe, and Leu, whereas non-essential amino acids are represented by Ala, Gly, Ser, Glu, Asp, Tyr, Cys, and Pro.

It has been suggested that in experiments estimating the ratio between an amino acid and lysine, care should be taken to make the latter secondary limiting dietary nutrient (Simongiovanni et al., 2012; Ajinomoto). These authors suggest a relative limitation of lysine of 10%. In the current experiment lysine was not limiting, so this could be another reason why no response of Gly was found. In a follow-up experiment of our group, the response of Gly supplementation will be tested under marginal lysine supplementation.

The growth performance results of the birds fed the low-protein diets was similar compared with the birds fed the positive control diet, which contained a 3% higher crude protein content but similar digestible EAA contents. These results are in line with the results of a previous study in our group (Van Harn et al., 2017).

The incidence of footpad lesions in the current study tended to be lower when low-protein diets were fed to the birds. This was in line with the results of a previous study where the footpad score decreased linearly with the decline of the dietary crude protein content (Van Harn et al. 2017). It is well-known that the most important factor causing FPD is considered to be wet litter (Shepherd and Fairchild, 2010). In the current study, the observed differences in litter quality are in line with the observed footpad scores, where broilers fed the control diet program had the worst litter quality (less friable and less dry) and the highest footpad scores.

In the present study feeding low-protein diets in general resulted in a decrease of the litter moisture content. All low-protein treatments numerically had a higher dry matter litter content than the control, but only 12.4 GLY, 14.9 GLY, 15.7 GLY and 12.4 GLY+THR diet programs differed significantly from the control. These results were in line with the findings of Kamran et al. (2010) and Ferguson et al. (1998a), but not completely in line with Moran et al. (1992), Elwinger and Svensson (1996), Ferguson et al. (1998b), Khajali and Moghaddam (2006) and Ospina-Rojas et al. (2012), who noted also a lower total nitrogen content but no change in the moisture content of the litter because of supplementing low-protein diets.

In general, lower dietary crude protein levels seem to reduce the N content of the litter (Elwinger and Svensson, 1996; Fergusson et al., 1998a,b; Khajali and Moghaddam, 2006 and Ospina-Rojas et al., 2012), and our results confirmed that as well. Ferguson et al. (1998a) reported that a 2% reduction in dietary crude protein decreased litter ammonium-N and nitrogen concentration by 31 and 16%, respectively. In this experiment the total nitrogen content and ammonium-N content of the litter of broilers fed the low-protein diets was 16% and 20% lower than the control group. Ospina-Rojas et al. (2012) found that birds fed diets with a CP reduction of 3% had 24% less N excretion via the litter and 37% ammonia emission, compared to birds fed a control diet.

5 Conclusions

From this experiment, in which the glycine+serine requirements and the possible glycine-sparing effect of threonine of male broilers from 10 – 35 days was studied, it can be concluded that:

Effect of glycine dose and glycine-sparing effect of threonine

- The dGly+Ser dose (5 levels - ranging from 12.4 to 15.7 g/kg and from 11.4 to 14.9 g/kg in grower and finisher phase, respectively) did not affect growth performance results.
- Broilers fed the diet program with the highest dGly+Ser dose had a lower breast meat yield compared with broilers who received 12.4 dGly+Ser and 13.3 dGly+Ser low-protein diet programs.
- Glycine dose had no effect on the litter quality and composition.
- Glycine dose had no effect on the severity of footpad lesions.
- Supplementing 0.7 g/kg extra dThr to low-protein diets did not have any beneficial effect on the growth performance results of male broilers. In this study, Thr did not demonstrate a Gly-sparing effect.

Effect of crude protein

- Providing broilers with diets that have approximately 3% lower crude protein but similar digestible AA-profile, in both the grower and finisher phase, resulted in similar growth performance results compared to broilers fed a diet program with a standard crude protein content.
- Compared to the control diet program none of the low-protein diet programs affected body weight gain, feed intake, feed efficiency or mortality from 0 – 35d negatively, but the crude protein efficiency improved significantly.
- Broilers fed the low-protein diet programs had, in general, dryer and more friable litter. This resulted towards a trend of a lower footpad score of broilers fed the low-protein diets.
- Broilers fed low-protein diets had a lower N-intake which resulted in less N-excretion and ammonium-N in the litter.

From this study, it can be concluded that dGly+Ser doses in low-protein diets of 12.4 g/kg and 11.4 g/kg in grower and finisher phase, respectively, are sufficient. It is also possible to reduce the crude protein content of grower and finisher diet with 30 g/kg, provided that the amino acid balance and level in the diets meet the bird's requirements by supplementation of free amino acids. By this, the soybean meal inclusion level can be reduced by 46%, relatively.

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Appendix 1 Lay-out of the experimental facility

12 Groen F	24 Rood A	36 Oranje G	48 Geel E	60 Zwart D	72 Grijs C
11 Oranje G	23 Grijs C	35 Blauw B	47 Blauw B	59 Oranje G	71 Blauw B
10 Rood A	22 Geel E	34 Geel E	46 Grijs C	58 Rood A	70 Rood A
9 Blauw B	21 Zwart D	33 Zwart D	45 Groen F	57 Zwart D	69 Zwart D
8 Grijs C	20 Geel E	32 Groen F	44 Geel E	56 Groen F	68 Geel E
7 Oranje G	19 Rood A	31 Groen F	43 Groen F	55 Blauw B	67 Groen F
6 Groen F	18 Oranje G	30 Oranje G	42 Rood A	54 Geel E	66 Oranje G
5 Geel E	17 Grijs C	29 Rood A	41 Zwart D	53 Grijs C	65 Rood A
4 Zwart D	16 Groen F	28 Grijs C	40 Blauw B	52 Groen F	64 Blauw B
3 Grijs C	15 Zwart D	27 Geel E	39 Oranje G	51 Grijs C	63 Grijs C
2 Blauw B	14 Oranje G	26 Blauw B	38 Zwart D	50 Rood A	62 Zwart D
1 Rood A	13 Blauw B	25 res	37 res	49 Oranje G	61 Geel E

Number 1, 2, 3, ...,72 = Pen number
Rood, Blauw, Grijs, Zwart. Geel, Groen and Oranje: different colours of the treatments.
Pen 25 and 37 (res): spare pens

Appendix 2 Composition and calculated nutrient contents of the starter and grower diets

		Starter	12.4GLY	13.3GLY	14.1GLY	14.9GLY	15.7GLY	12.4GLY +7.5THR	Control
Wheat	%	30.000	30.000	30.000	30.000	30.000	30.000	30.000	30.000
Corn	%	27.432	38.730	38.730	38.730	38.730	38.730	38.730	28.163
Soybean meal HiPro nGMO	%	29.104	15.797	15.797	15.797	15.797	15.797	15.797	25.924
Rapeseed meal 00	%	3.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Soyalecithin	%	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Soya oil	%	2.249	1.619	1.619	1.619	1.619	1.619	1.619	3.504
Lauric fatty acids	%	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Limestone	%	0.270	0.039	0.039	0.039	0.039	0.039	0.039	0.010
L-Lysine HCl	%	0.226	0.486	0.486	0.486	0.486	0.486	0.486	0.168
Sodiumbicarbonate	%	0.302	0.389	0.389	0.389	0.389	0.389	0.389	0.237
DL-methionine	%	0.271	0.312	0.312	0.312	0.312	0.312	0.312	0.221
Sodiumchloride	%	0.097	0.007	0.007	0.007	0.007	0.007	0.007	0.116
L-threonine	%	0.089	0.196	0.196	0.196	0.196	0.196	0.196	0.049
L-Threonine (on top)	**%**	--	--	--	--	--	--	**0.070**	--
L-Valine 98	%	0.069	0.204	0.204	0.204	0.204	0.204	0.204	0.023
Xylanase 6,25%	%	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Axtra Phy 5000L	%	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Oat hulls	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
monocalciumphosphate	%	0.981	0.770	0.770	0.770	0.770	0.770	0.770	0.664
L-Arginine	%	--	0.304	0.304	0.304	0.304	0.304	0.304	0.008
L-Isoleucine	%	--	0.178	0.178	0.178	0.178	0.178	0.178	--
L-Glycine (on top)	**%**	--	--	**0.088**	**0.170**	**0.252**	**0.334**	--	--
Tryptofaan	%	--	0.059	0.059	0.059	0.059	0.059	0.059	0.003
VM VLK 20-40	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
VM VLK 0-20 dgn	%	--	--	--	--	--	--	--	--
VM Maxiban 0,3%	%	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
AMEn broiler	kcal/kg	2900	3000	3000	3000	3000	3000	3000	3000
Crude protein	g/kg	219	177	177	177	177	177	177	209
Crude fat	g/kg	65	61	61	61	61	61	61	78
Crude fibre	g/kg	35	34	34	34	34	34	34	36
crude ash	g/kg	54	44	44	44	44	44	44	49
Starch Brunt	g/kg	359	430	430	430	430	430	430	363
Calcium	g/kg	8.0	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Phosphorus	g/kg	6.2	5.4	5.4	5.4	5.4	5.4	5.4	5.5
Sodium	g/kg	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium	g/kg	9.6	7.2	7.2	7.2	7.2	7.2	7.2	9.2
dEB (Na+K-Cl)		259	192	192	192	192	192	192	243
6-Phytase E4a24	ftu	500	500	500	500	500	500	500	500
Dig.lysine	g/kg	11.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Dig.methionine	g/kg	5.5	5.4	5.4	5.4	5.4	5.4	5.4	5.0
Dig.met+cys	g/kg	8.5	7.9	7.9	7.9	7.9	7.9	7.9	7.9
Dig.threonine	**g/kg**	7.5	**6.8**	**6.8**	**6.8**	**6.8**	**6.8**	**7.5**	**6.8**
Dig.tryptophan	g/kg	2.38	2.30	2.30	2.30	2.30	2.30	2.30	2.30
Dig.isoleucine	g/kg	7.8	7.4	7.4	7.4	7.4	7.4	7.4	7.4
Dig.valine	g/kg	9.2	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Dig.arginine	g/kg	12.5	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Dig.glycine + serine	**g/kg**	16.5	**12.44**	**13.30**	**14.10**	**14.90**	**15.70**	**12.44**	**15.70**
Dig. P broiler	g/kg	4.4	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Narasin	mg/kg	50	50	50	50	50	50	50	50
Nicarbazin	mg/kg	50	50	50	50	50	50	50	50

Appendix 3 Composition and calculated nutrient contents of the finisher diets

		12.4GLY	13.3GLY	14.1GLY	14.9GLY	15.7GLY	12.4GLY +7.5THR	Control
Wheat	%	30.000	30.000	30.000	30.000	30.000	30.000	30.000
Corn	%	43.024	43.024	43.024	43.024	43.024	43.024	31.748
Soybean meal HiPro nGMO	%	12.560	12.560	12.560	12.560	12.560	12.560	23.363
Rapeseed meal 00	%	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Soyalecithin	%	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Soya oil	%	1.049	1.049	1.049	1.049	1.049	1.049	3.061
Lauric fatty acids	%	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Limestone	%	0.277	0.277	0.277	0.277	0.277	0.277	0.246
L-Lysine HCl	%	0.509	0.509	0.509	0.509	0.509	0.509	0.170
Sodiumbicarbonate	%	0.395	0.395	0.395	0.395	0.395	0.395	0.244
DL-methionine	%	0.303	0.303	0.303	0.303	0.303	0.303	0.206
Sodiumchloride	%	0.015	0.015	0.015	0.015	0.015	0.015	0.124
L-threonine	%	0.201	0.201	0.201	0.201	0.201	0.201	0.044
L-Threonine (on top)	**%**	--	--	--	--	--	**0.080**	--
L-Valine 98	%	0.212	0.212	0.212	0.212	0.212	0.212	0.018
Xylanase 6,25%	%	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Axtra Phy 5000L	%	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Oat hulls	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Monocalciumphosphate	%	0.268	0.268	0.268	0.268	0.268	0.268	0.155
L-Arginine	%	0.324	0.324	0.324	0.324	0.324	0.324	0.009
L-Isoleucine	%	0.191	0.191	0.191	0.191	0.191	0.191	--
L-Glycine (on top)	**%**	--	**0.084**	**0.173**	**0.259**	**0.351**	--	--
Tryptofaan	%	0.062	0.062	0.062	0.062	0.062	0.062	0.002
VM VLK 20-40	%	--	--	--	--	--	--	--
VM VLK 0-20 dgn 8754	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000
VM Maxiban 0,3%	%	--	--	--	--	--	--	--
AMEn broiler	kcal/kg	3025	3025	3025	3025	3025	3025	3025
Crude protein	**g/kg**	**165**	**165**	**165**	**165**	**165**	**165**	**199**
Crude fat	g/kg	57	57	57	57	57	57	75
Crude fibre	g/kg	34	34	34	34	34	34	36
crude ash	g/kg	39	39	39	39	39	39	43
Starch Brunt	g/kg	458	458	458	458	458	458	386
Calcium	g/kg	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Phosphorus	g/kg	4.1	4.1	4.1	4.1	4.1	4.1	4.28
Sodium	g/kg	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium	g/kg	6.5	6.5	6.5	6.5	6.5	6.5	8.6
dEB (Na+K-Cl)		175	175	175	175	175	175	231
6-Phytase E4a24	ftu	500	500	500	500	500	500	500
Dig.lysine	g/kg	9.9	9.9	9.9	9.9	9.9	9.9	9.9
Dig.methionine	g/kg	5.5	5.5	5.5	5.5	5.5	5.5	4.7
Dig.met+cys	g/kg	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Dig.threonine	**g/kg**	**6.4**	**6.4**	**6.4**	**6.4**	**6.4**	**7.1**	**6.4**
Dig.tryptophan	g/kg	2.17	2.17	2.17	2.17	2.17	2.17	2.17
Dig.isoleucine	g/kg	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Dig.valine	g/kg	7.9	7.9	7.9	7.9	7.9	7.9	7.9
Dig.arginine	g/kg	11.3	11.3	11.3	11.3	11.3	11.3	11.3
Dig.glycine + serine	**g/kg**	**11.41**	**12.29**	**13.16**	**14.00**	**14.90**	**11.41**	**14.90**
Dig. P broiler	g/kg	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Narasin	mg/kg	--	--	--	--	--	--	--
Nicarbazin	mg/kg	--	--	--	--	--	--	--

Appendix 4 Visual litter scoring method

Litter is visually scored on friability and moisture per pen according to the scoring scale below by three persons independently.

Friability	
1	(1- friable litter)
2	(2- 25% of the area is caked)
3	(3- 50% of the area is caked)
4	(4- 75% of the area is caked)
5	(5- complete caked litter)
Moisture	
1	(1- dry litter)
2	(2- slightly moist litter)
3	(3- moist litter)
4	(4- wet litter)
5	(5- extremely wet litter)

Gradations of the moisture

- Dry litter which is often only observed at start. Maybe a bit brown coloured but particles do not stick to each other
- Slightly moist litter has particles sticking to each other a bit, and litter can be a bit darker coloured.
- Moist litter result in a ball-shaped form when pressed but will fall apart afterwards.
- Wet litter shows a feet pattern if pressed on the litter but no moist will be visually pressed outside
- Extremely wet litter is soggy and shows moist flowing outside the litter when pressing it.

Appendix 5 Overview of scores for foot-pad lesions

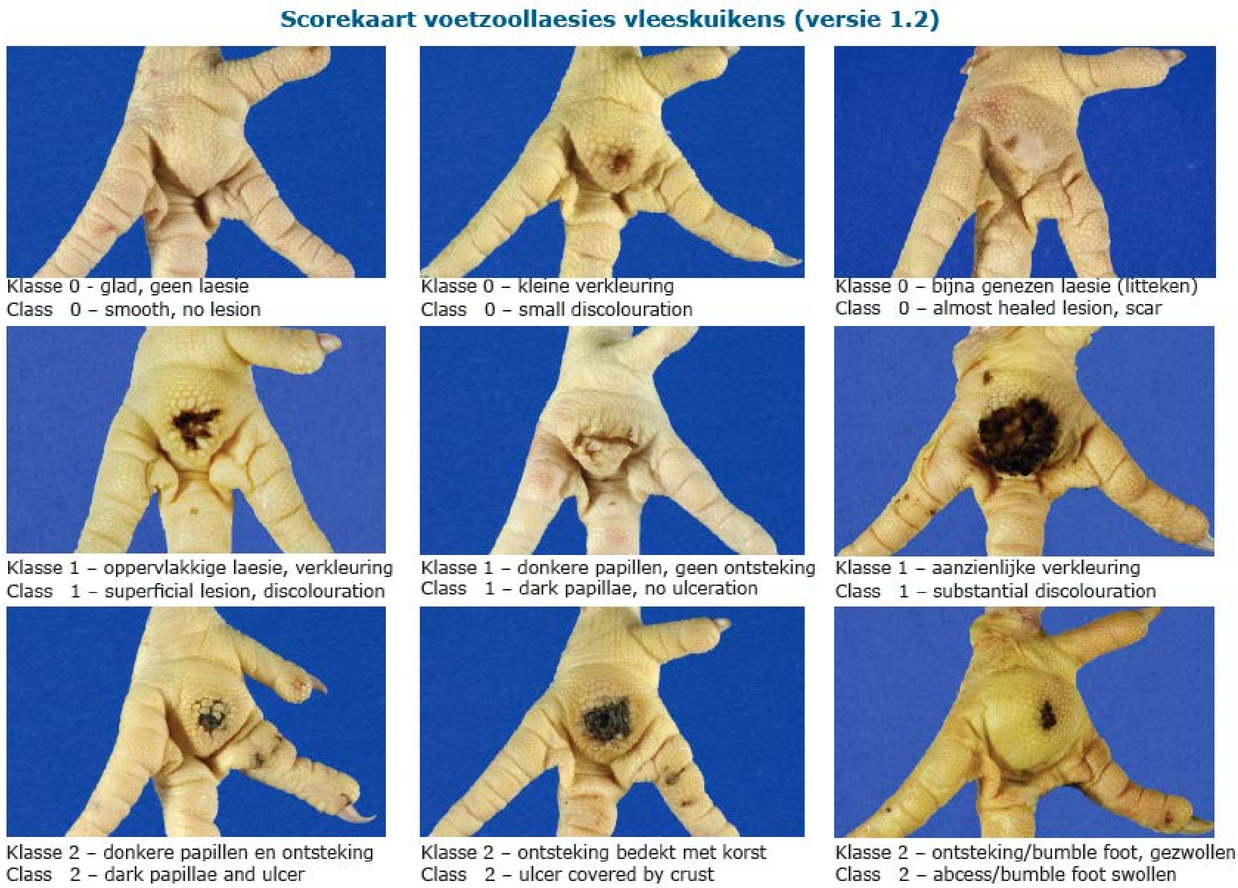

Table A5.1 *Footpad score, average score and % per scoring class.*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
FPS	41.0^{B}	68.4^{AB}	62.0^{AB}	44.5^{B}	43.2^{B}	46.1^{B}	101.1^{A}	0.057	14.66
Avg. score	0.64^{b}	0.93^{ab}	0.79^{b}	0.57^{b}	0.66^{b}	0.72^{b}	1.29^{a}	0.027	0.15
% Score 0 footpads	44.4^{ab}	29.6^{ab}	42.9^{ab}	59.4^{a}	44.7^{ab}	38.5^{ab}	7.2^{b}	0.011	9.32
% Score 1 footpads	46.7^{ab}	48.3^{ab}	34.7^{ab}	24.4^{a}	44.9^{ab}	51.3^{ab}	56.3^{a}	0.037	6.95
% Score 2 footpads	8.8	22.1	22.3	16.2	10.4	10.2	36.5	0.119	7.40

[a,b] Values without a common superscript per row differ significantly ($P < 0.05$).

[A,B] Values without a common superscript indicate a tendency ($0.05 \leq P < 0.10$).

[1] According to Berg (1998); Class 0 – no lesions, class 2 – mild lesions and class 2 – severe lesions. [2] FPS = (%Sc1*0.5) + (%Sc2*2); range 0 (all animals score 0) – 200 (all animals score 2).

Appendix 6 Growth performance from 0–10 and 0–28 days of age

Table A6.1 *Growth performance results from 0 – 10 days (starter phase).*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Body weight (g)	274	275	273	280	274	270	278	0.463	3.5
Body weight gain (g)	234	235	233	240	234	230	238	0.463	3.5
Growth (g/d)	23.4	23.5	23.3	24.0	23.4	23.0	23.8	0.463	0.35
Mortality (%)	1.5	0.0	0.0	0.0	0.0	0.8	0.0	0.182	0.49
Feed conversion ratio	1.082	1.118	1.128	1.100	1.105	1.097	1.116	0.669	0.0210
Feed intake (g)	252	262	263	264	258	252	265	0.254	5.1
Feed intake (g/d)	25.3	26.2	26.3	26.4	25.8	25.2	26.5	0.254	0.51
CP intake (g)	55.3	57.7	57.9	58.1	56.8	55.3	58.4	0.254	1.12
CP conversion (g/g)	0.237	0.246	0.248	0.242	0.243	0.241	0.245	0.669	0.0046

^{a,b} Values without a common superscript per row differ significantly (P < 0.05).

Table A6.2 *Growth performance results from 0 – 28 days (starter + grower phase).*

	12.4 GLY	13.3 GLY	14.1 GLY	14.9 GLY	15.7 GLY	12.4 GLY + THR	Control	F-prob	SEM
Body weight (g)	1446	1434	1500	1497	1463	1424	1474	0.524	32.1
Body weight gain (g)	1406	1394	1460	1457	1423	1384	1434	0.524	32.1
Growth (g/d)	50.2	49.8	52.2	52.0	50.8	49.4	51.2	0.524	1.15
Mortality (%)	5.4	1.5	0.8	3.8	1.5	2.3	1.7	0.144	1.24
Feed conversion ratio	1.557	1.555	1.497	1.502	1.521	1.566	1.490	0.551	0.0351
Feed intake (g)	2172	2161	2180	2184	2159	2159	2122	0.738	26.9
Feed intake (g/d)	77.6	77.2	77.8	78.0	77.1	77.1	75.8	0.738	0.96
CP intake (g)	395.3 ^{b}	401.5 ^{b}	404.8 ^{b}	403.8 ^{b}	402.8 ^{b}	393.0 ^{b}	438.9 ^{a}	<.001	5.01
CP conversion (g/g)	0.282 ^{ab}	0.289 ^{ab}	0.278 ^{b}	0.278 ^{b}	0.284 ^{ab}	0.285 ^{ab}	0.308 ^{a}	0.033	0.0066

^{a,b} Values without a common superscript per row differ significantly (P < 0.05).

To explore the potential of nature to improve the quality of life

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