Effect of low protein diets supplemented with free amino acids on growth performance, slaughter yield, litter quality, footpad lesions, economical performance and the ecological footprint of male broilers

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J. van Harn[1], M.A. Dijkslag[2] and M.M. van Krimpen[1]

[1] Wageningen Livestock Research
[2] ForFarmers

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Samenvatting
Het verstrekken van voeders met een verlaagd eiwitgehalte aangevuld met vrije aminozuren om de aminozuurbehoefte van vleeskuikens te dekken kan bijdragen tot het verminderen van het gebruik van sojaschroot van Zuid Amerikaanse herkomst. Verondersteld werd dat het verstrekken van voeders met een lager eiwitgehalte de carbon footprint kan verlagen doordat minder soja in het voer verwerkt hoeft te worden. Vanuit de literatuur is bekend dat het verlagen van het eiwitgehalte in het voer, zonder dat hierbij essentiële aminozuren worden aangevuld, resulteert in een verslechtering van de dierprestaties (groei en voerefficiëntie). Het aanvullen van vleeskuikenvoeders met een verlaagd ruw eiwitgehalte met vrije aminozuren is noodzakelijk om de productieresultaten op peil te houden. Vleeskuikenvoeders worden normaliter geoptimaliseerd op minimale eisen voor verteerbaar lysine, methionine+cysteine, threonine en tryptofaan. Bij laag eiwit voeder kunnen echter ook de aminozuren valine, isoleucine, serine en glycine beperkend worden. In dit rapport worden de resultaten beschreven van een vleeskuikenstudie waarin de effecten van het verstrekken van groei- en afmestvoeders met een 10, 20 of 30 gram per kilogram verlaagd ruw eiwitgehalte ten opzichte van een controlerantsoen met een eiwitgehalte in het groei- en afmestvoer van 208 en 198 g/kg, maar met een vergelijkbaar aminozuurgehalte op de productieresultaten, slachtrendementen, strooiselkwaliteit, voetzoollaesies, economische resultaten en carbon footprint werd onderzocht.

Summary
Providing diets with a reduced crude protein (CP) content that are supplemented with increased contents of free amino acids (AA) to cover the AA requirement might be helpful in reducing the soybean meal content of South American origin, and thus in reducing the amount of non-EU protein in the diet. For maintaining performance levels of broilers that are fed low CP diets, the AA supply is essential. Broiler diets are usually at least optimised for minimum requirements of digestible lysine, methionine + cysteine, threonine and tryptophan. In low CP diets, the requirements for the AA valine, isoleucine, serine and glycine should also be taken into account as well. The impact of using diets with a low CP content and a high amount of free AA content on the ecological footprint is not known.
This report describes the results of an experiment with male broilers in which the effect of low CP diets, with a partial replacement of soybean meal by free AA, on technical performance, slaughter yields, litter quality, footpad lesions, economical performance and the ecological footprint was evaluated.

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Foreword

Feed4Foodure is a public-private partnership between the Dutch Ministry of Economic Affairs, 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 soybean meal content of South American origin, and thus reducing the amount of non-EU protein in the diet of pigs and poultry.
The current report describes the results of an experiment with male broilers in which the effects of low crude protein diets, with a partial replacement of soybean meal by free amino acids, on growth performance, slaughter yields, litter quality, footpad lesions, economical performance and the ecological footprint were evaluated. For the current study, scientists of Wageningen UR Livestock Research worked together with representatives from the consortium. The authors thank the industry partners of the project team for their worthwhile 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 their AA requirements might be helpful in reducing the use of soybean meal of South American origin, and thus in reducing the amount of non-EU protein in broiler diets. For maintaining performance levels of broilers that are fed low CP diets, the AA supply is essential. Broiler diets are usually at least optimised for minimum lysine, methionine + cysteine, threonine and tryptophan requirements. In low CP diets, the requirements for the AA valine, isoleucine, serine and glycine should be taken into account as well.

A study was conducted in which the effects of low CP diets, with a partial replacement of soybean meal by free AA, on technical performance, slaughter yields, litter quality, footpad lesions, economical performance and the ecological footprint were evaluated. In this study, dietary soybean meal content in the grower diets was reduced from 27.3% (control) to 17.3% (CP-3%), while soybean meal content in the finisher diets was reduced from 25.0% (control) to 14.6% (CP-3%). 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 68 were used for this study. All pens were bedded with white wood shavings and housed 13 male broilers. The lighting schedule was 23 h d^{-1} from 0 to 2d, 18 h d^{-1} from 3 to 35d, and the temperature was gradually decreased from 34 ^{o}C to 20 ^{o}C at 34d of age.
Broilers received a 3-phase diet programme: starter (0 to 11d), grower (11 to 28d) and finisher (28 to 35d of age). All broilers received the same starter diet, and thereafter four feeding programmes with different levels of dietary CP (control, and three low protein diets with 1% (CP-1%), 2% (CP-2%) and 3% (CP-3%) less CP per period) were tested. The control diet programme had had a crude protein content in the grower and finisher phase of 208 and 198 g/kg, 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.

Reducing dietary CP content up to 3%, while maintaining dietary concentrations of essential AA resulted in similar growth performance or even better growth performance results as broilers fed the control diet program. The best overall performance was obtained with the CP-2% diet program.
Over the entire experimental period (0 – 35d) none of the low protein diet programs affected body weight gain, feed intake or mortality. However, the crude protein efficiency improved with the decline of the crude protein content of the diet. Broilers fed the CP-1% diet program had numerically but not significantly a better feed conversion ratio compared to the control fed birds. Broilers fed the CP-2% or CP-3% diet program had a statistically improved feed conversion ratio.
Broilers fed the low protein diet programs had a lower water intake, a better litter quality and less severe footpad lesions, thus a lower footpad score compared to broilers fed the control diet program.

Providing broilers diets with up to a 3% lower crude protein content while maintaining dietary concentrations of essential AA hardly influenced the slaughter yields. Only the breast meat yield, expressed as % of the carcass, of broilers fed the diet with 3% lower CP content was lower, while the breast meat weight did not differ.

Lowering the crude protein content substantially reduced the inclusion of non-European protein (soybean meal, SBM) in the diets, but increased the calculated dietary carbon footprint as well the feed price. A 1% lower crude protein content reduced the amount of SBM with 2.5 percent, but the carbon footprint and feed price increased with 6 and 3 percent, respectively. A 3% reduction of the crude protein content reduced the SBM content with 10.2, and increased the carbon footprint and feed price with respectively with 19 and 10 percent. Low crude protein diets had, despite the better growth performance results, a lower feed margin at farm level. Compared with the control CP group the feed margin per broiler placed of the CP-1%, CP-2% and CP-3% was 2.6, 2.4 and 6.1 €cent lower, respectively.

1   Introduction

The world population is growing and the demand for animal products is increasing (FAO, 2006). It is expected that the demand for poultry meat will grow rapidly worldwide, and thus also the demand for plant proteins, e.g. soybean meal, will increase. The accessibility of plant proteins for feed is expected to decline with an increasing demand for more highly digestible diet ingredients to be used for human consumption rather than for inclusion in animal diets. In Europe, the rate of self-sufficiency for soybean meal, however, is only 2% (FEFAC, 2015), indicating the extreme dependency for this important protein source on other areas, mainly South America. The European Parliament is concerned that such a massive dependency on imports makes the EU livestock sector extremely vulnerable to price volatility and trade distortions, causing feed price to rise, thereby increasing farmers' production costs and reducing the sectors' profitability (Euractiv, 2011). A major concern of NGO's is the deforestation of tropical rain forest, to fulfil the need of arable land for soybean cultivation (WNF, 2011; Van Gelder and Kuepper, 2012). As a consequence of conversion of natural ecosystems into agriculture, the rate of biodiversity loss (proportion of extinct species) increases, whereas the current status has already more than ten times exceeded the proposed boundary (Rockström et al., 2009). Moreover, large scale soybean cultivation may increase water and soil pollution, and drive small farmers and the native population out of business (WNF, 2011). It is, therefore, important to find alternatives for imported soybean meal.

A lower inclusion of soybean protein in broiler diets is one way to reduce the use of soybean in Europe. This can be done by partial replacing soy products by other protein commodities as: peas, rapeseed / rapeseed meal, sunflower meal, fish meal, corn gluten meal, and / or potato protein. Soybean meal has a balanced amino acids pattern that meets the requirements of most of the amino acids of poultry, and a complete replacement of soybean meal by the aforementioned protein-rich feed stuffs, with a less balanced pattern of amino acids, will result in an increase of the use of free amino acids. Partial replacement of soybean meal by free amino acids seems, in view of the economic aspects, is more obvious.

The provision of diets with a lower crude protein content was suggested to reduce the carbon footprint, because less of soybean meal in the feed needs to be transported and processed. Reducing the crude protein content of broiler diets, however, will increase the risk of reduced growth performance, when (semi-) essential amino acids become limiting. In order to successfully reduce crude protein in broiler feeds, thus to maintain growth performance results, it is essential to keep the supply and the balance of most limiting amino acids in line with the broiler requirement through an adequate dietary supplementation of free amino acids. Findings in literature show that, as long as contents of lysine and the ratio of the other essential amino acids to lysine are maintained, low protein diets allow to achieve maximum level of performance (Waldroup et al., 1976; Kerr and Kidd, 1999; Aletor et al., 2000; Dean et al., 2006; Namroud et al., 2008). However, in low protein diets also glycine and serine, that currently are considered as non-essential amino acids, (Dean et al., 2006) can become growth limiting, either because there are not enough metabolic precursors for the respective amino acids available, or because endogenous metabolization processes are too slow (Aftab et al., 2006; Berres et al., 2010). This could well be the reason why several researchers found decreased performance results with low protein diets supplemented with amino acids, without maintaining the glycine + serine to lysine ratio (e.g. Ferguson et al., 1998a,b; Bregendahl et al, 2002).
When the crude protein content is reduced, glycine and serine levels decrease as well (Dean et al., 2006). Therefore, it is possible that marginal levels of dietary glycine and serine are the reason for the decrease in performance of broilers when feeding low protein diets, even if they are supplemented with essential amino acids. Several studies showed that glycine supplementation avoid the adverse effects on broiler performance when low protein diets were provided (Ospina-Rojas et al., 2012; Ospina-Rojas et al., 2013; Ospina Rojas et al., 2014). In a study of Veldkamp et al. (2016, in press) with broiler chickens from 8–35 days of age, reduced growth performance results were found of

broilers fed low protein diets, despite essential amino acids (lysine, methionine, tryptophan, threonine, arginine and isoleucine) were supplemented to 10% above CVB (2012) requirements. The authors suggested that the lack of glycine and serine might explain the reduced growth performance results in low protein diets. Because of the importance of glycine in low-protein diets, it is considered as the fourth limiting amino acid after lysine, methionine and threonine (Waguespack et al., 2009). Also according to Ospina-Rojas (2013), glycine is a limiting amino acid in low protein diets, especially in the starter phase but also afterwards. Based on the obtained results in the study of Veldkamp et al. (2016) and in the literature, it is decided in the present study to add free glycine to low protein diets, aiming to maintain the production results of broilers.

A large part of the dietary nitrogen intake is not retained by the animal, but excreted into the environment. Lowering the crude protein content of the diet could therefore be a tool to reduce the nitrogen excretion and ammonia emission from broiler houses (Elwinger and Svensson, 1996; Van Harn and Van Middelkoop, 1996; Kidd et al., 1996; Ferguson et al., 1998a; Nahm, 2002; Khajali and Moghaddam, 2006; Namroud, et al., 2008; Hernandez et al., 2013). Moreover, low crude protein levels in broiler diets reduce the risk of digestion problems and necrotic enteritis. A reduced crude protein would also lead to less water intake, because there is a reduced need to get rid of the protein surplus (Bailey, 1999; Elwinger and Svensson, 1996). A lower water intake reduces the risk of wet litter, and thus risk of an impaired welfare since wet litter is the main reason for skin dermatitis such as footpad lesions, hock burns and breast blisters. Wet litter could also lead to more rejections at the slaughter house (Shephard and Fairchild, 2010).

Besides the fact that providing low protein diets to broilers reduces the use of soybean meal, it could also by an efficient tool to improve intestinal health (less digestive disorders and improved health status), animal welfare (less dermatitis) and to reduce environmental emissions (less N-excretion and ammonia emission) (Qaisrani et al., 2015; Apajalahti and Vienola, 2016).

An experiment was performed with male broilers in which the effects of feeding low crude protein diets, with an 1%, 2% or 3% reduced crude protein content but supplemented with free amino acids (including glycine), thereby partial replacing soybean meal, on growth performance, slaughter yields, litter quality, litter composition and animal welfare were studied. Also the consequences of adjusting the dietary protein content of the diets on the feed costs and carbon footprint of the diets were calculated.

2 Materials and Methods

2.1 Study objective

The objective of this study was to evaluate the effects of low crude protein diets, with a partial replacement of soybean meal by free amino acids, on growth performance, slaughter yields, litter quality, footpad lesions, economical performance and the ecological footprint.
Low crude protein diets were supplemented with free amino acids and provided from 11 – 35 days of age.

2.2 Start date and end date of the experiment

Start date: 7 January 2016
End date: 11 February 2016

2.3 Experimental animals

A total of 936 day-old male Ross 308 broiler chicks were used and allocated to 72 floor pens bedded with wood shavings (13 animals/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 (VB 3358) and age of the parent stock was 36 weeks.

2.4 Experimental design

The experiment was carried out at the experimental broiler house of feed producer ForFarmers. This natural ventilated house locates 72 floor pens of 0.75 m^2, of which 68 were used for this study. A completely randomized block design, consisting of four treatments and seventeen blocks, was used in this experiment. A block consisted of 4 consecutive pens (Appendix 1). Each treatment was replicated seventeen times and the experimental unit was a pen with 13 male broilers (Table 2.1). Treatments were randomly allotted among pens. Randomization was conducted within blocks to create a balanced distribution of treatments within blocks, and over the experimental room taking into account front and backside of the facility. The treatments and a description of the treatments are presented in Table 2.2.

Table 2.1 *Experimental design in summary.*

Item	Number
Treatments	4
Broilers per pen	13
Broilers per treatment	221
Total number of broilers	884
Number of blocks	17
Replicates (pens) per treatment	17
Total number of pens	68

Table 2.2 *Experimental treatments.*

Treatment	Description
1	Control, standard crude protein (CP)
2	Control – 1% CP; diet with a 1% lower CP, but similar digestible amino acids (dAA) [1] to control
3	Control – 2% CP; diet with a 2% lower CP, but similar dAA [1] to control
4	Control – 3% CP; diet with a 3% lower CP, but similar dAA [1] to control

[1] Lysine, methionine, threonine, valine, arginine, isoleucine, glycine and tryptophan.

2.5 Experimental diets

The experimental diets were formulated by ForFarmers and produced by Research Diet Services, Wijk bij Duurstede, The Netherlands. A three-phase diet program was provided. Starter, grower and finisher diets were provided from 0 – 11, 11 - 28 and 28 – 35 days of age, respectively. During the starter phase all animals receive the same diet with crude protein content of 216 g/kg. Thereafter, four diet programmes with different crude protein levels (control, and three low protein programmes with 1% (CP-1%), 2% (CP-2%) and 3% (CP-3%) less CP per period) but similar essential digestible amino acids levels were provided. The control diet programme had had a crude protein content in the grower and finisher phase of 208 and 198 g/kg, respectively. The diets were formulated to meet or exceed the requirements concerning faecal digestible AA (lysine, methionine, threonine, tryptophan, isoleucine, valine, arginine and glycine), and to be iso-caloric within each period. All diets for each period were prepared with the same batch of ingredients. Dietary soybean meal content in the grower diets reduced from 27.3% (control) to 17.3% (CP-3%), while soybean meal content in the finisher diets reduced from 25.0% (control) to 14.6% (CP-3%). The ingredient and nutrient composition (incl. AA ratios relative to lysine) were established in consultation with the F4F project partners (Table 2.3).

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

		Starter	Grower diets				Finisher diets			
		Contr.	Contr.	-1%	-2%	-3%	Contr.	-1%	-2%	-3%
AMEn broiler	kcal/kg	2900	3000	3000	3000	3000	3025	3025	3025	3025
Crude protein	g/kg	216	208	198	188	178	198	188	178	168
Dig. lysine	g/kg	11.5	10.5	10.5	10.5	10.5	9.9	9.9	9.9	9.9
Dig. methionine	g/kg	5.6	5.0	5.1	5.3	5.5	4.7	4.9	5.1	5.2
Dig. meth+cys	g/kg	8.5	7.9	7.9	7.9	7.9	7.5	7.5	7.5	7.5
Dig. threonine	g/kg	7.5	6.8	6.8	6.8	6.8	6.4	6.4	6.4	6.4
Dig. tryptophan	g/kg	2.37	2.30	2.30	2.30	2.30	2.17	2.17	2.17	2.17
Dig. isoleucine	g/kg	7.8	7.4	7.4	7.4	7.4	7.0	7.0	7.0	7.0
Dig. valine	g/kg	9.2	8.4	8.4	8.4	8.4	7.9	7.9	7.9	7.9
Dig. arginine	g/kg	12.5	12.0	12.0	12.0	12.0	11.3	11.3	11.3	11.3
Dig. glycine + serine	g/kg	16.3	15.7	15.7	15.7	15.7	14.9	14.9	14.9	14.9

The ingredient composition and the complete calculated nutrient contents of all diets are presented in Appendix 2. In Appendix 3 the AA ratio to lysine is provided per feeding phase.
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 (pellet temperature < 70 ºC).

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). The floor pens were located in the natural ventilated poultry house of J.W. van Essen, Nijkerk, The Netherlands. Sixty eight of these 72 pens were used for this trial. In the remaining four pens, reserve 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 –

11d). These reserve animals were fed the same starter diet. Therefore, the number of animals per pen was similar for all pens at day 11 (= start providing experimental diets). Per pen 13 broilers were placed. The housing, management, feeding and husbandry conditions are standard and representative for a modern commercial broiler operation in Europe.

Water and feed were provided *ad libitum* 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 30°C. The temperature at placement of the broilers was 34°C and this temperature was decreased gradually to 20°C at 34 days of age (Table 2.4). During the first two days light was nearly continuous on (23L:1D), from 3 – 35 days of age a day/night schedule of 18h light and 6h dark (18L:6D) was given 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 day-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, 4 grower and 4 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 Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

❑ Performance
Body weight (BW) of birds per pen was determined at 0, 11, 28 and 35 days of age. Feed intake (FI) per pen was determined at 11, 28 and 35 days of age (provided feed minus remaining feed at 11, 28 and 35 days of age). Body weight gain (BWG), average daily gain (ADG), feed conversion ratio (FCR), feed intake (FI), average daily feed intake (ADFI) were calculated on a pen basis from these data for the following periods: 0 – 11d, 11 – 28d, 28 – 35d, 11 – 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).
Water intake was measured, but not per pen but per treatment per two rows. As the feed intake per pen is known, the cumulative feed intake per treatment in these two rows could be calculated. By dividing cumulative water intake by cumulative feed intake, the average water/feed ratio per treatment in these two rows was calculated. By multiplying the average water/feed ratio per two rows and the feed intake per pen the water intake per pen could be estimated.
Twice daily, in the morning and the afternoon, animals and housing facilities were inspected by the farmer, thereby checking the general health status, continuous supply of feed and water as well as lighting, temperature and ventilation, and recognizing unexpected events. All incidences were recorded.
If an animal was in poor condition, health status was observed more frequently. 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 35 days of age by an experienced assessor, who scored the friability and wetness of the litter in each pen on a 1 to 10 point scale. The scores and the description of each score are presented in Table 2.5.

Table 2.5 *Scores for friability and wetness of the litter and a description of each score.*

Score	Friability description	Wetness description
1	Complete caked litter	Wet litter, total area by pressure on the litter water is appearing
2	80-90 % of the area is caked	Wet litter, beneath drinking line by pressure on the litter water is appearing
3	70-80 % of the area is caked	Wet litter, beneath drinking line by pressure on the litter no water is appearing
4	60-70 % of the area is caked	Wet litter, dark coloured. Litter can be pressed ball-shaped.
5	50-60 % of the area is caked	Wet litter, dark coloured. Ridges beneath drinking line
6	40 % of the area is caked	Almost dry litter, small ridges beneath drinking line. Litter between drinking line and feeders is still friable
7	30 % of the area is caked	Almost dry litter, dark coloured beneath drinking line and in other areas light coloured, ridge formation beneath drinking lines just started
8	10 % of the area is caked	Almost dry litter, light coloured, no ridges beneath drinking line
9	Friable litter, some litter particles are caked	Dry litter, light coloured
10	Friable litter, no caked litter particles	Very dry litter

❑ Litter composition

At the end of the study representative samples of the litter were taken from pens 13 – 60. These samples were analysed on: dry matter, total N, ammonium N and pH. These analyses were done 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 footpad lesions is presented in Appendix 4.

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 ranges from 0 (all birds having no lesions) to 200 (all birds having score 2).

❑ Economic calculations

An economic calculation was performed based on obtained growth performance results and slaughter yield results obtained in the current experiment. Prices were based on KWIN-V 2016-2017 and Bedrijfswijzer Pluimvee (described by Vellinga et al. (2013) and Vermeij and Kanis (2005)).

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 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 LSD (least significant difference (P=0.05)) 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:

$Y_{ij} = \mu + Treatment_i + Error_{ij}$

Where:

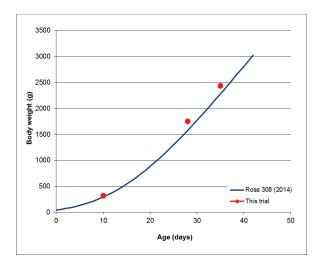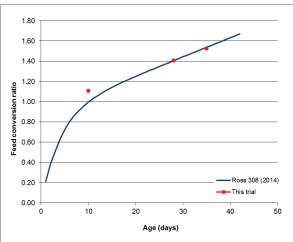|	
Y	Response parameter
μ	General mean
Treatment	Effect of diet (i=1…4)
Error	Error term

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 37.6 grams. Overall mortality during the experiment was 5.1%, which is slightly higher than the average Dutch standard (3.5%, KWIN 2016/2017). For this experiment, however, only male broilers were used and it is well known that the mortality of male broilers is higher than of female broilers.

Average body weight at 35 days was 153 gram higher (2436 vs. 2283 grams), and average feed conversion ratio over the entire experimental period (0 – 35 days) was slightly lower (1.526 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) achieved in this experiment compared with the Ross 308 broiler male performance objectives (Aviagen, 2014)*

3.2 Diet analyses

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

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

Nutrient		Starter diet	Grower diets				Finisher diets			
			Control CP	CP-1%	CP-2%	CP-3%	Control CP	CP-1%	CP-2%	CP-3%
Crude protein (calc)	g/kg	216	208	198	188	178	198	188	178	168
Crude protein.(ana)	g/kg	207	200	194	186	178	191	184	176	168
Crude fat (calc)	g/kg	67	83	76	70	64	79	71	66	60
Crude fat (ana)	g/kg	68	81	76	70	66	75	71	66	61
Crude fibre (calc)	g/kg	35	37	36	35	34	35	35	34	34
Crude fibre (ana)	g/kg	34	37	37	35	36	37	38	38	34
Crude ash (calc)	g/kg	54	48	47	46	44	43	42	40	39
Crude ash (ana)	g/kg	51	47	46	45	42	42	40	39	36
Starch Brunt (calc)	g/kg	353	351	373	397	421	376	399	421	445
Starch Brunt (ana)	g/kg	347	350	364	385	404	359	379	407	432

From this table, it can be concluded that the crude protein level of the starter diet was relatively 4.2% lower than calculated. Crude protein level of the control grower and finisher diets were respectively 3.8 and 3.5 % lower than calculated. The differences between analysed and calculated crude protein decreased with decreasing crude protein. Although the analysed differences in crude protein content were lower than calculated (the absolute difference in crude protein content between control and CP-3% was 2.2% and 2.3% for grower and finisher diet, respectively, instead of 3%) the differences in crude protein content between treatments were still in line with the experimental set-up. The analysed starch content of most diets, except grower control, was lower than calculated. On average the difference in calculated and analysed starch content was 3%. Analysed crude fat, crude fibre and crude ash content met the calculated values.

Table 3.2 *Calculated and analysed amino acids contents of the grower and finisher diets*

		Grower diets				Finisher diets			
		Control CP	CP-1%	CP-2%	CP-3%	Control CP	CP-1%	CP-2%	CP-3%
Calculated									
Lysine	g/kg	11.8	11.7	11.6	11.5	11.1	11.1	11.1	10.8
Methionine	g/kg	5.4	5.5	5.6	5.8	5.1	5.2	5.4	5.5
Cysteine	g/kg	3.5	3.4	3.2	2.9	3.4	3.3	3.1	2.8
Threonine	g/kg	8.1	8.0	7.9	7.8	7.6	7.6	7.5	7.4
Tryptophan	g/kg	2.6	2.4	2.2	2.1	2.4	2.2	2.0	2.0
Isoleucine	g/kg	8.4	8.3	8.2	8.1	7.9	7.8	7.8	7.7
Arginine	g/kg	13.2	12.8	12.7	12.6	12.4	12.1	12.0	11.9
Phenylalanine	g/kg	9.8	9.2	8.4	7.8	9.3	8.7	7.9	7.3
Histidine	g/kg	5.3	5.0	4.6	4.2	5.0	4.7	4.3	4.0
Leucine	g/kg	15.7	15	13.9	13	15.2	14.3	13.3	12.3
Tyrosine	g/kg	6.6	6.2	5.6	5.1	6.2	5.8	5.3	4.8
Valine	g/kg	9.7	9.7	9.6	9.5	9.2	9.2	9.1	9.0
Alanine	g/kg	9.1	8.7	8.1	7.6	8.8	8.4	7.8	7.2
Asparagine	g/kg	19.3	17.9	16.1	14.6	18.1	16.6	14.7	13.5
Glutamic acid	g/kg	39.7	37.6	34.9	32.3	38	35.8	33.1	30.6
Glycine	g/kg	8.6	8.2	8.7	9.2	8.2	7.7	8.1	8.5
Proline	g/kg	12.6	12.2	11.5	10.8	12.3	11.8	11.1	10.3
Serine	g/kg	9.8	9.2	8.5	7.8	9.3	8.7	7.9	7.3
Analysed									
Lysine	g/kg	11.5	11.6	11.5	11.3	10.7	10.8	10.7	10.7
Methionine	g/kg	5.1	5.2	5.4	5.5	4.9	5.0	5.1	5.3
Cysteine	g/kg	3.3	3.2	3.1	2.9	3.3	3.2	3.0	2.8
Threonine	g/kg	8.0	8.1	7.8	7.7	7.4	7.4	7.3	7.3
Tryptophan	g/kg				Not determined				
Isoleucine	g/kg	8.6	8.5	8.4	8.0	8.0	8.0	7.8	7.8
Arginine	g/kg	13.2	13.2	13.0	12.7	12.3	12.2	12.0	12.0
Phenylalanine	g/kg	10.0	9.6	8.7	8.0	9.4	9.0	8.1	7.5
Histidine	g/kg	5.1	5.0	4.5	4.1	4.7	4.6	4.2	3.8
Leucine	g/kg	15.6	15.4	14.2	13.1	14.7	14.6	13.3	12.6
Tyrosine	g/kg				Not determined				
Valine	g/kg	9.9	9.9	9.5	9.3	9.4	9.2	9.0	8.9
Alanine	g/kg	9.1	9.0	8.3	7.8	8.5	8.6	7.9	7.4
Asparagine	g/kg	19.3	18.3	16.2	14.5	17.7	16.9	14.8	13.3
Glutamic acid	g/kg	39.6	37.5	34.2	31.5	37.6	35.1	32.0	29.9
Glycine	g/kg	8.7	9.0	9.5	9.9	8.0	8.5	9.0	9.5
Proline	g/kg	12.6	12.3	11.4	10.8	12.1	11.6	10.8	10.3
Serine	g/kg	9.8	9.4	8.5	8.0	9.2	8.8	7.9	7.4

In Table 3.2 the calculated and analysed amino acids (AA) contents of the grower and finisher diets are given. Appendix 5 presented the relative differences (in %) between calculated and analysed amino acids contents of the grower and finisher diets.

It can be concluded that in general the analysed AA contents met the calculated contents. It is noteworthy that when free glycine was added, a strong increase of the analysed glycine content is analysed. This increase of the glycine content in the analysis must be related to glycine present in protein in the ingredients, because the amount of supplemented free glycine was close to the formulated inclusion levels. The former excludes a dosing error of glycine.

3.3 Growth performance results

This paragraph describes the growth performance results obtained in the grower phase (11 -28 days), finisher phase (28 – 35 days), grower and finisher phase (11 – 35 days) and the overall experimental period (0 – 35 days). In Appendix 6 the obtained growth performance results from 0 – 11 days (starter phase) of age are given.

3.3.1 Grower phase

In Table 3.3, the growth performance results from 11 – 28 days of age are given. From these results it can be concluded that providing low protein diets supplemented with free AA including glycine had no negative effect on the performance results compared to the control, a grower diet with 208 g/kg dietary protein.

Body weight (BW), body weight gain (BWG), mortality, feed conversion ratio (FCR) and feed intake were not affected by any of the low protein treatments. However, the crude protein conversion decreased with the decline of the crude protein content of the diets, which means that broilers fed the diet with the lowest crude protein had the highest protein efficiency. The water intake and water/feed ratio decreased with the decrease of the crude protein content of the feed.

Table 3.3 *Performance results per treatment from 11 - 28 days of age (grower phase)*

	Control CP	CP-1%	CP-2%	CP-3%	F-prob	LSD
Body weight 11d (g)	324	323	323	321	0.656	4.3
Body weight 28d (g)	1752	1751	1752	1739	0.759	29.1
Body weight gain (g)	1428	1428	1429	1417	0.811	27.7
Body weight gain (g/d)	84.0	84.0	84.1	83.4	0.811	1.63
Mortality (%)	3.2	3.6	2.3	3.6	0.834	3.39
Feed conversion ratio (g/g)	1.456	1.468	1.443	1.458	0.553	0.0340
Crude protein conversion (g/g)	0.3029 [a]	0.2906 [b]	0.2713 [c]	0.2595 [d]	<.001	0.0064
Feed intake (g/d)	122.3	123.2	121.3	121.5	0.566	2.92
Water intake (ml/d)	227.2 [a]	217.5 [b]	206.4 [c]	199.4 [d]	<.001	4.98
Water/Feed ratio (ml/g)	1.86	1.77	1.70	1.64		

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

3.3.2 Finisher phase

As in the grower phase, during the finisher phase (28 – 35 days), no adverse effects of lowering the crude protein content of the diet were found on BW, BWG, mortality and FI (Table 3.4). Broilers fed the CP-3% diet had a better (lower) FCR compared to broilers fed the control diet or the CP-1% diet. Broilers fed the CP-2% diet had also a better FCR than the control fed broilers, but the FCR did not differ from the broilers who received CP-1% or CP-3% diets. The FCR of the broilers fed the CP-1% diet did not differ from the control fed birds. As in the grower phase the crude protein conversion, water intake and water/feed ratio linearly decreased with the decrease of the crude protein of the diet. Unlike the grower phase, the water intake of the broilers fed the CP-2% diet did not differ from those who received CP-3% diet.

Table 3.4   Performance results per treatment from 28 - 35 days of age (finisher phase)

	Control CP	CP-1%	CP-2%	CP-3%	F-Prob	LSD
Live weight (g)	2416	2431	2447	2448	0.595	53.4
Body weight gain (g)	666	685	697	708	0.143	37.0
Body weight gain (g/d)	95.2	97.9	99.6	101.1	0.143	5.29
Mortality (%)	2.6	1.5	0.5	1.0	0.458	2.69
Feed conversion ratio (g/g)	1.954 [a]	1.886 [ab]	1.801 [bc]	1.772 [c]	<.001	0.0806
Crude protein conversion	0.3868 [a]	0.3546 [b]	0.3206 [c]	0.2977 [d]	<.001	0.0154
Feed intake (g/d)	184.2	183.8	179.0	179.1	0.175	6.25
Water intake (ml/d)	341.2 [a]	316.3 [b]	296.9 [c]	294.1 [c]	<.001	10.85
Water/Feed ratio (ml/g)	1.85	1.72	1.66	1.64		

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

3.3.3   Grower and finisher phase

In Table 3.5 the performance results from 11 - 35 days of age were given. In this period the broilers received the experimental grower (11 – 28d) and finisher diets (28 – 35d).

From 11 – 35 days of age dietary treatments did not alter BWG or FI. Broilers fed the control diet had the poorest FCR and broilers fed the CP-2% diet the best. FCR of broilers fed the CP-1% diet did not differ from the FCR of birds fed the control or the CP-3% diets. Broilers fed CP-2% or CP-3% diets had improved FCR compared with the birds fed the control diets. Broilers fed the CP-3% diets had the best crude protein conversion, followed by broilers fed the CP-2%, CP-1% and control diets, respectively. The water intake decreased with the decrease of the crude protein content of the diet. Broilers fed the control diets had a higher water intake than birds who received CP-1% diet. Whereas broilers fed the CP-1% had a higher water intake than birds fed CP-2%, which had a similar water intake as birds fed the CP-3% diet program. Dietary treatment did not influence the incidence of mortality.

Table 3.5   *Performance results per treatment from 11-35 days (grower and finisher phase)*

	Control CP	CP-1%	CP-2%	CP-3%	F-prob	LSD
Live weight (g)	2416	2431	2447	2448	0.595	53.4
Body weight gain (g)	2092	2109	2125	2127	0.528	52.8
Body weight gain (g/d)	87.2	87.9	88.5	88.6	0.528	2.20
Mortality (%)	5.9	4.8	2.4	4.8	0.393	4.20
Feed conversion ratio (g/g)	1.610 [a]	1.601 [ab]	1.558 [c]	1.563 [bc]	0.002	0.0307
Crude protein conversion	0.3288 [a]	0.3109 [b]	0.2870 [c]	0.2722 [d]	<.001	0.0059
Feed intake (g/d)	140.3	140.6	137.9	138.4	0.147	3.46
Water intake (ml/d)	260.4 [a]	245.9 [b]	232.4 [c]	227.2 [c]	<.001	4.84
Water/Feed ratio (ml/g)	1.86	1.75	1.69	1.64		

[a,b,c,d] 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.

From 0 – 35 days of age dietary treatments did not alter BW at 35d, BWG, mortality or FI. However FCR, crude protein conversion, and water intake were affected by dietary treatment. As from 11 – 35d, broilers fed CP-2% or CP-3% diets had an improved FCR compared with the birds fed the control diet program, which had the poorest FCR. FCR of broilers fed the CP-1% diet did not differ from broilers fed the control or the CP-3% diet program, but the FCR was poorer than broilers fed CP-2% diet program.

Table 3.6 *Performance results per treatment from 0 – 35 days of age (= complete experimental period)*

	Control CP	CP-1%	CP-2%	CP-3%	F-prob	LSD
Live weight (g)	2416	2431	2447	2448	0.595	53.4
Body weight gain (g/d)	68.0	68.4	68.8	68.9	0.595	1.53
Mortality (%)	6.0	5.8	2.5	6.3	0.324	4.72
Feed conversion ratio (field) (g/g)	1.549 [a]	1.542 [ab]	1.505 [c]	1.510 [bc]	0.002	0.0265
Crude protein conversion	0.3178 [a]	0.3023 [b]	0.2814 [c]	0.2685 [d]	<.001	0.0051
Feed intake (g/d)	105.2	105.4	103.6	103.9	0.147	1.93
Water intake (ml/d)	196.6 [a]	186.6 [b]	177.4 [c]	173.8 [c]	<.001	3.32
Water/Feed ratio	1.87	1.77	1.71	1.67		
European Efficiency Factor (EPEF)	413 [b]	417 [b]	447 [a]	428 [ab]	0.035	24.5
Footpad score (FPS)	143 [a]	110 [b]	79 [c]	39 [d]	<.001	28.1

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

In line with the results from 11 – 35d, the protein conversion decreased with the decline of the crude protein content of the diet program. Broilers fed the CP-3% diets had the best crude protein conversion, followed by broilers fed the CP-2%, CP-1% and control diet program, respectively. Water intake decreased with the decrease of the crude protein content of the diet. However, the difference in water intake between broilers fed the CP-2% diet program and those who received the CP-3% diet program was not significant. As the feed intake was similar for all treatments, and the water intake was decreased with the decline of the crude protein content the water to feed ratio also decreased with the decrease of the crude protein. Broilers fed the CP-2% diet program had the highest European Efficiency Factor (EPEF), which means that this treatment had the best overall performance results. The EPEF of the birds who received the CP-1% or CP-3% diet program did not differ from the birds in the control group.
Broilers fed the control diet program had the highest footpad score. This means that broilers of this group had the highest incidence and the most severe footpad lesions. The footpad score decreased with the decline of the crude protein content of the diet. The incidence of footpad lesions was in-line with the litter quality (see 3.5).

3.4   Slaughter yields

In Table 3.7 the slaughter yields per treatment on 35 days of age are given.

Table 3.7 *Slaughter yields per treatment at day 35 of age*

	Control CP	CP-1%	CP-2%	CP-3%	F-prob	LSD
Live weight (LW, g)	2446	2481	2488	2495	0.250	51.4
Carcass weight (CW, g)	1615	1645	1648	1647	0.145	33.1
Carcass (% of LW)	66.0	66.3	66.2	66.0	0.402	0.46
Wing (g)	166	169	168	167	0.270	3.3
Wing (% of CW)	10.3 [a]	10.3 [a]	10.2 [ab]	10.1 [b]	0.027	0.12
Leg (g)	547 [b]	555 [ab]	557 [ab]	564 [a]	0.035	11.3
Leg (% of CW)	33.9 [b]	33.8 [b]	33.8 [b]	34.3 [a]	0.028	0.33
Back (g)	243	246	251	253	0.051	7.3
Back (% of CW)	15.1 [b]	15.0 [b]	15.2 [ab]	15.3 [a]	0.028	0.27
Breast meat (Filet) (g)	520 [ab]	532 [b]	526 [ab]	513 [a]	0.046	13.5
Breast meat (Filet) (% of CW)	32.1 [ab]	32.3 [a]	31.9 [b]	31.2 [c]	<.001	0.39

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

Providing broilers low protein diets had no significant effect on live weight, carcass weight or carcass yield. However, it did affect the wing, leg, back and breast meat yield. Broilers fed the CP-3% diets program had a lower wing and breast meat yield but a higher leg and back yield compared with the control group. However, no differences between Control CP and CP-3% were found in the weights of the wing, back and breast meat. Male broilers fed the CP-3% diet program had a higher leg weight

(thigh and drum) than those who received the control diet program. No differences in slaughter yields were found between broilers fed CP-1% or CP-2% and the control fed broilers. Although relative breast meat yield (% of CW) reduced with decreasing crude protein content, the absolute amount of breast yield (g) of the low crude protein treatments was not lower compared to the control diet program.

3.5　Litter quality and composition

In Table 3.8 the visual litter quality (friability and wetness) and the litter composition at 35 day age is given. It can be concluded that the litter quality increased, indicating more friable and dryer litter, when the crude protein content of the diet decreased. Broilers fed the CP-3% diet program had the best (visual) litter quality, followed by CP-2% and CP-1% diet program, and broilers fed the control diet program had the worst litter quality. No significant differences were found in visual litter quality between CP-1% and CP-2%.

Table 3.8　*Visual litter quality and dry matter, total nitrogen, ammonium nitrogen and pH of the litter on 35 days of age*

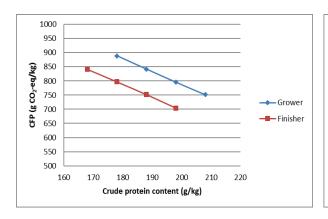|	Control CP	CP-1%	CP-2%	CP-3%	F-prob	LSD
Friability [1]	3.3 [c]	4.2 [b]	4.7 [b]	6.1 [a]	<.001	0.79
Wetness [2]	2.7 [c]	3.7 [b]	4.4 [b]	5.8 [a]	<.001	0.82
Dry matter (g/kg)	387 [b]	401 [ab]	404 [ab]	436 [a]	0.033	32.7
Total-N (g/kg DM)	43.7 [a]	42.6 [a]	40.8 [ab]	38.2 [b]	0.002	2.78
Ammonia-N (g/kg DM)	11.3 [ab]	13.3 [a]	12.7 [ab]	11.1 [b]	0.026	1.65
pH	6.5	6.8	7.1	7.0	0.122	0.53

[1] 1 – 10: 1 completely caked – 10: completely friable; [2] 1 -10: 1 very wet – 10 dry

The results of the visual litter quality correspond reasonably well with the dry matter (DM) contents of the litter. Broilers fed with the CP-3% diet program had the highest DM-content of the litter. However, the DM-content of the CP-3% group did only differ from the control, and not from the CP-1% and CP-2% group.

Total-N of the litter of the control fed birds was significantly higher than CP-3%, which means that more N was excreted. The total-N of the litter of CP-1% and CP-2% fed animals was in between and did not differ from either the control or the CP-3% group. Litter ammonium-N content of broilers fed the CP-3% was significantly lower than CP-1% fed broilers, but it did not significantly differ from the control and CP-2% fed groups. No differences between dietary treatments were found in pH of the litter.

3.6　Effect of low protein diets on economic parameters and carbon footprint

In Figure 3.2 the effect of crude protein content of the diet on the carbon foot print and price of the feed is given. In Table 3.9 and 3.10, the revenues on farm (feed margin) and the revenues of chain (carcass value slaughter, based on the yield and prices of the different carcass parts) are provided, respectively. In appendix 7 the feed composition is given as well as the contribution of the individual feedstuffs used in the experimental diets on the carbon footprint. Data were generated from the FeedPrint database. The CFPAN tool and database FeedPrint calculates the carbon footprint of feed raw materials during their complete life cycle. This ranges from crop production, via processing of crop and animal products, compound feed production to utilization by the animal, including transport and storage between all steps of the production chain. For feed prices the prices of the feed ingredients from January 2017 were taken.
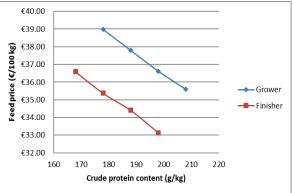
Figure 3.2 Effect of crude protein content of the experimental diets on the carbon foot print (left) and feed price (right) of the diets.

From this figure it can be concluded that a decrease of the crude protein content of broiler diets resulted in an increase of the feed costs as well in an increase of the carbon footprint (CFP). Each 0.8 percent lower crude protein leads to an increase of the CFP with 6%, and to a 3% higher feed price.

Broilers fed the control diet program had the highest feed margin (Table 3.9) and broilers fed the CP-3% had the lowest feed margin. The feed margin of the broilers who received CP-1% or the CP-2% diet program were in between. The CP-3% group had a 6.1 €cents per broiler delivered lower feed margin than the control group. The difference in feed margin between the CP-1% and CP-2% group with the control group was 2.6 and 2.4 €cents per delivered broiler, respectively.

Table 3.9 *Feed margin at farm level per treatment at day 35 of age.*

		Control CP	CP-1%	CP-2%	CP-3%
Live weight	g	2416	2431	2447	2448
Mortality		3.5	3.5	3.5	3.5
FCR		1.549	1.542	1.505	1.51
Amount of starter feed consumed	g	315	315	315	315
Amount of grower feed consumed	g	2079	2094	2062	2066
Amount of finisher feed consumed	g	1289	1287	1253	1253
Meat price € per kg live weight [1]	€/kg	0.835	0.835	0.835	0.835
Day-old chicken price [1]	€/piece	0.335	0.335	0.335	0.335
Feed prices					
Starter[2]	€/100 kg	34.00	34.00	34.00	34.00
Grower[2]	€/100 kg	32.00	32.93	33.99	35.05
Finisher[2]	€/100 kg	30.00	31.16	32.04	33.14
Revenue per bird	€	2.017	2.030	2.043	2.044
Chicken price	€	0.347	0.347	0.347	0.347
Feed cost per bird	€	1.159	1.198	1.210	1.247
Feed margin	€/bird	**0.511**	**0.485**	**0.487**	**0.450**

[1] KWIN 2016-2107. [2] Feed prices of the control CP treatment are based on KWIN 2016-2017, whereas additional feed costs for 1%, 2% and 3% CP reduction are added to the feed prices of the control CP treatment.

The higher feed price of the low crude protein diets was related to the extra supplementation of free amino acids in these diets. Lowering the crude protein content of the grower diet with 1, 2 or 3% increased the feed price with 2.9, 6.2 and 9.5%, respectively. For the finisher diet this was 3.9, 6.8 and 10.5%, respectively. The improved performance of the broilers fed the CP-2% or CP-3% diet programs could not compensate for the increased feed costs, overall resulting in lower feed margins of these groups compared with the control fed broilers.

In Table 3.10, the revenues of chain are provided, taking into account the economic value of the wings, legs, back and breast meat. Providing low protein diets, supplemented with free amino acids, to broilers resulted in similar (CP-3%) or even higher (in case of CP-1% or CP-2%) chain results. Broilers fed the control diet program had a similar chain revenue compared to broilers who received CP-3% diet program (€4.388 vs. €4.392). Compared with the CP-1% and CP-2% groups, the chain result of the control group was respectively 8.9€cents and 6.0€cents lower. This difference was largely attributable to the higher leg and breast meat weight of the broilers fed the CP-1% or CP-2% diet programs.

Table 3.10 *Revenues of chain per treatment at day 35 of age.*

		Control CP	CP-1%	CP-2%	CP-3%
Live weight	g	2446	2481	2488	2495
Carcass weight	g	1615	1645	1648	1647
Wing	g	166	169	168	167
Leg	g	547	555	557	564
Back	g	243	246	251	253
Breast meat (Filet)	g	520	532	526	513
Wing [1]	€/kg	1.50	1.50	1.50	1.50
Leg [1]	€/kg	2.25	2.25	2.25	2.25
Back [1]	€/kg	0.20	0.20	0.20	0.20
Breast meat [1]	€/kg	5.50	5.50	5.50	5.50
Wing	€	0.249	0.254	0.252	0.251
Leg	€	1.231	1.249	1.253	1.269
Back	€	0.049	0.049	0.050	0.051
Breast meat	€	2.860	2.926	2.893	2.822
Total	€/bird	**4.388**	**4.477**	**4.448**	**4.392**

[1] BedrijfsWijzer Pluimvee

4 Discussion

Providing broilers with low protein diets often results, even if they were supplemented with free amino acids, in declined performance results (e.g. Ferguson et al., 1998a,b; Bregendahl et al, 2002). However, the growth performance results in the present study of the broilers fed the AA supplemented low protein diet programs were similar or even better compared with broilers fed the control diet program.

In the present experiment the obtained growth performance results of broilers fed the low protein diets were better than for the control fed broilers. This phenomenon has not often been observed. Mostly, similar or deteriorated growth performance is observed with low-protein diets. A deteriorated litter quality and a higher incidence of footpad lesions, consequently resulting in less locomotion and reduced feed intake, not observed in the present trial, of the control fed birds, thermal discomfort, could be reasons of the decreased growth performance results of the control fed birds. De Jong et al. (2014) found decreased growth performance results of birds kept on wet litter and had a high incidence of severe footpad lesions. Broilers kept on dry and friable litter had better growth performance results than those who were kept on wet litter.

Besides, it is well known that high-protein diets might have negative effects on gut health and performance (Apajalahti and Vienola, 2016; Qaisrani et al, 2015). Ileal bypass protein is subject to fermentation by putrefactive bacteria in the caecum. Putrefaction produces many harmful and toxic compounds, which in high concentrations may have adverse effects on chicken growth and performance. The protein fermentation products include toxic components like amines, indoles, phenols, cresol and ammonia, which in high concentrations may have adverse effects on chicken growth and performance (Apajalahti and Vienola, 2016). Reduction of ileal bypass protein potentially also reduce production of toxic protein fermentation metabolites in the caeca.

Moreover, the inclusion of free glycine in the diets in the present study, thereby covering the glycine and serine requirement, may be a solid explanation of the obtained similar (CP-1% and CP-3% diet program) or even better (CP-2% diet program) growth performance results of the low protein groups. According to Ospina-Rojas et al. (2013) supplemental glycine may be essential to support maximum performance for broiler chickens when they are fed diets based on vegetable ingredients and with low protein levels.

Broiler chickens are constantly in contact with the litter. It is well known that increased litter moisture increases the risk for skin dermatitis (footpad lesions, hock burns and dermatitis of the skin on the chest), e.g. Greene et al. (1985) and Martland (1985). In addition, broilers suffering from pain caused by (severe) footpad dermatitis (Martland, 1985) and possibly also from dermatitis on the hocks and breast may be less eager to go to the feeders and drinkers and thus may show less weight gain as broilers having no or mild footpad dermatitis. De Jong et al. (2014) found that wet litter not only induces contact dermatitis (like footpad lesions, hock burns, breast blisters), it had also negative effects on birds locomotion, growth performance results and carcass yield. According to De Jong et al. (2014) the deteriorated growth performance results were caused by the fact that birds having severe footpad lesions (and possibly also from hock burns and/or breast dermatitis) walk less, because they suffer from pain. As a result of this they may be less eager to go to the feeders and drinkers. Also wet litter feels cold which resulted in more huddling and thermal discomfort. It could well be that the deteriorated growth performance results of broilers fed the control diet were caused by the deteriorated litter quality. Broilers of the control group suffer more from footpad lesions compared to broilers fed the low protein diet programs. Also the deteriorated litter quality in the control group may also have led to some thermal discomfort of the birds, which may also have affected their growth performance results (especially FCR) negatively.

The incidence of footpad lesions in the current study decreased linearly with the decline of the dietary crude protein content. 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 good in line with the found footpad scores. Broilers fed the control diet program had the worst litter quality (less friable and less dry) and the highest footpad scores.

Reducing dietary crude protein resulted in a decrease of the litter moisture content and nitrogen content. 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 also noted a decrease in the nitrogen content, but no change in the moisture content of the litter. Ferguson et al (1998a) found with a 2% reduction in dietary crude protein a decreased litter ammonia and nitrogen concentrations of 31 and 16.5%, respectively. In this trial the total nitrogen content of the low protein diets were only 2.5%, 6.6% and 12.6% lower than the control group, for CP-1%, CP-2% and CP-3% respectively. However, only the N-content of the litter of the CP-3% fed broilers differed significantly from the control. The measured reduction in total nitrogen in this trial was lower than in the study of Ferguson et al (1998a). In this trial the provision of low protein diets did not result in a reduction of the ammonium N content of the litter. This was not in-line with the expectation. It was expected that providing low protein diets to broilers would result in a lower ammonium nitrogen content of the litter, but in this trial none of the low protein groups had a significant lower ammonium-N compared with the control group. In general, lower dietary crude protein levels seen 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). Our results confirm that, even in a multiphase feeding program, the excretion of N was decreased with reduced levels of dietary crude protein.

As expected, the reduction in dietary crude protein content affected the daily water intake of the broilers and consequently the DM content of the litter. The reduction in water consumption by lowering the dietary crude protein content is a well-known effect (Bailey, 1999). A higher protein intake increases the amount of metabolites, e.g. uric acid and potassium, that have to be excreted in urine, and water is necessary to excrete nitrogenous waste products from the protein metabolism. Because of the lower water intake in the low-protein diets fed birds, also the water/feed ratio decreased, which was in line with findings of Elwinger and Svensson (1996). Despite the lower water intake of the broilers fed the low protein diets, the litter dry matter was not influenced. Although the findings regarding water intake met our expectations, it should be noticed that water intake was not measured per pen but per treatment per two rows of pens, indicating that no hard conclusion can be drawn from the observed lower water / feed ratio. However, feeding low protein diets does not always affect litter moisture content. Elwinger and Svensson (1996), Ferguson et al. (1998b), Hernandez et al. (2013) and Ospina-Rojas et al. (2014) did not find any effect in their studies on litter moisture content. Differences in season, housing and management (in particular ventilation) could explain absence of effects on litter moisture content.

In the current study, no effects on the litter acidity or pH of the litter were detected for the dietary crude protein content, which is in agreement with previous studies (Elwinger and Svensson, 1996; Ferguson et al., 1998b; Hernandez et al., 2013 and Ospina-Rojas et al., 2014). However, in contrast to our study, Ferguson et al (1998a) reported a decrease in litter pH with low protein diets.

Broilers fed the CP-3% diets program had a lower wing and breast meat yield but a higher leg and back yield compared with the control group. However, due to the higher body weight at slaughter age the weight of the different parts did not differ from the control fed broilers. Feeding AA supplemented diets with up to two percent lower crude protein did not affect the slaughter yields. These results are in line with Ospina-Rojas et al (2014), who also found no differences in slaughter yields (and growth performance results) in diets with a 3% reduction in crude protein AA supplemented diets. According to Ospina-Rojas it is important that glycine and arginine is added to low protein diets, accompanied by the supplementation of valine and isoleucine, besides lysine, methionine and threonine, to maintain adequate slaughter yields.

Despite the lower SBM content in the low protein diets the carbon foot print (CFP) was not lower compared to the control diet. The use of free amino acids, especially threonine, is the main reason for the higher CFP. For example the CFP for free L-lysine, DL-methionine, L-tryptophan, L-arginine, L-isoleucine, L-threonine is 6030, 5490, 9500, 9500, 9500 and 16978 g CO_2-eq/kg, respectively (FeedPrint database). Marinussen and Kool (2010) describe the environmental impact of the production of free amino acids L-lysine, D,L-methionine and L-threonine. For the production of L-threonine, high quantities of sulphate compounds are required, which significantly contribute to the acidification of the environment. This explains the very high CFP value of L-threonine.

5 Conclusions

From this study, in which in the grower and finisher phase (11 – 35d) diets with a 1, 2 or 3% lower crude protein content but similar digestible amino acids contents of the first nine limiting amino acids including glycine were provided, thereby reducing the soybean meal content up to 10%, it can be concluded that:

Providing broilers diets with up to a 3% lower crude protein content but similar digestible AA in the grower and finisher phase resulted in similar growth performance or even better growth performance results compared to broilers fed a control diet program with a dietary protein content in the grower and finisher phase of 208 and 198 g/kg, respectively. The best overall performance results were obtained with the CP-2% diet program.
Over the entire experimental period (0 – 35d) none of the low protein diet programs affected body weight gain, feed intake or mortality. However, the crude protein efficiency improved with the decline of the crude protein content of the diet. Broilers fed the CP-1% diet program had numerically but not significantly a better feed conversion ratio compared to the control fed birds. Broilers fed the CP-2% or CP-3% diet program had a statistically improved feed conversion ratio.
Broilers fed the low protein diet programs had a lower water intake, which resulted in a better litter quality and less severe footpad lesions, thus a lower footpad score.

Providing broilers diets with up to a 3% lower crude protein content but similar digestible AA hardly influenced the slaughter yield. Only the breast meat yield of broilers fed the diet with 3% lower crude protein content was lower, while the breast meat weight did not differ.

Providing low protein diets resulted in an increase of the carbon footprint as well in an increase of the feed price, whereas feed margin was reduced in the low crude protein diets.

Overall it can be concluded that supplementation of free AA to the diet allows to reduce soybean meal content of broiler diets by 10%, thereby reducing crude protein content. Feeding broilers diets with a 3% lower crude protein content did not result in adverse effects on growth performance results and slaughter yields if they are supplemented, with sufficient lysine, and in the recommended ratio to lysine, with free methionine, threonine, arginine, isoleucine, valine and glycine. The former could be a possible measure to reduce the amount / share of non-European protein in the broiler diets. However, replacing (partly) non-European protein by European protein in combination with an increased use of free amino acids will increase the feed costs and carbon foot print and reduce feed margin.

How further?
Currently, free glycine is not registered for use as free amino acid, which hampers the implementation of low crude protein diets in broiler production. Threonine (Thr) is a known precursor of glycine (Gly) via the Thr dehydrogenase and aldolase pathways (Davis and Austic, 1982). It was demonstrated before that Thr could spare the Gly requirement (Ospina-Rojas et al., 2013; Siegert et al., 2015). In an upcoming trial, the responses of broilers to graded levels of Gly in the diets with respect to growth performance, slaughter yields, litter quality and footpad lesions will be investigated in low crude protein diets. In addition, in this study the glycine sparing effect of threonine will be evaluated.

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Appendix 1 Lay-out of the experimental facility

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

Number 1, 2, 3, ...,72 = Pen number
Rood, Blauw, Grijs en Zwart: different colours of the treatments.
Pen 1R, 2R, 3R en 4R: reserve pens

Appendix 2 Feed composition and calculated nutrients of the diets

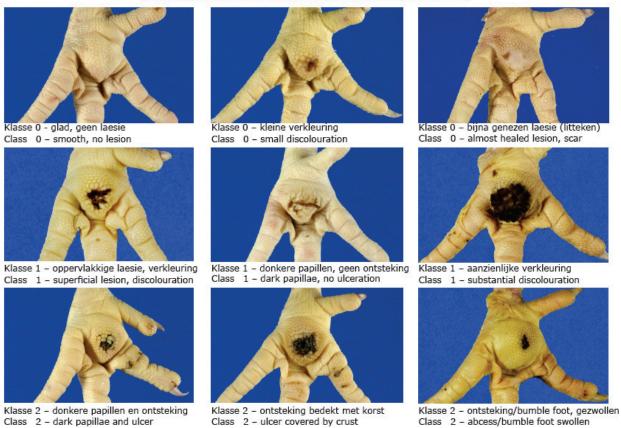|		Starter diet	Grower diets				Finisher diets			
			Contr	-1%	-2%	-3%	Contr	-1%	-2%	-3%
Raw materials										
Wheat	%	30.87	37.29	30.00	30.00	30.00	39.64	30.00	30.00	30.29
Corn	%	25.00	18.80	29.04	32.75	36.51	20.56	33.02	36.49	40.00
Soybean meal HiPro nGMO	%	30.47	27.34	24.84	21.07	17.30	24.99	22.55	18.41	14.62
Rapeseed meal 00	%	3.00	5.00	4.96	5.00	5.00	4.28	4.40	5.00	5.00
Palmoil crude	%	1.54	2.71	2.45	2.19	1.51	2.51	2.25	1.68	1.01
Soya lecithin	%	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Soya oil	%	0.92	1.51	0.87	0.50	0.50	1.22	0.50	0.50	0.50
Lauric fatty acids	%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	%	0.25	--	--	--	--	0.25	0.25	0.25	0.26
L-Lysine HCl	%	0.22	0.16	0.24	0.36	0.48	0.16	0.24	0.36	0.47
Sodium bicarbonate	%	0.28	0.21	0.25	0.31	0.39	0.24	0.28	0.34	0.41
DL-methionine	%	0.28	0.23	0.25	0.29	0.32	0.21	0.24	0.27	0.31
Sodium chloride	%	0.11	0.13	0.10	0.06	--	0.12	0.09	0.05	--
L-threonine	%	0.09	0.05	0.09	0.14	0.19	0.05	0.08	0.14	0.19
L-Valine 98	%	0.07	0.02	0.07	0.14	0.21	0.02	0.07	0.14	0.20
Xylanase 6,25%	%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phyzyme XP 5000L	%	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Oat hulls	%	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mono calcium phosphate	%	0.99	0.66	0.69	0.76	0.82	0.15	0.19	0.22	0.26
L-Arginine	%	--	--	0.08	0.19	0.30	--	0.08	0.19	0.30
L-Isoleucine	%	--	--	0.04	0.11	0.18	--	0.04	0.11	0.18
L-Glycine	%	--	--	0.09	0.21	0.34	--	0.09	0.22	0.34
Tryptophan	%	--	--	0.02	0.04	0.06	--	0.02	0.04	0.06
VM VLK 0-20 dgn 8754	%	2.00	2.00	2.00	2.00	2.00	--	--	--	--
VM VLK 20-40 dgn.	%	--	--	--	--	--	2.00	2.00	2.00	2.00
VM Maxiban 0,3%	%	0.30	0.30	0.30	0.30	0.30	--	--	--	--
Nutrients										
AMEn broiler	kcal/kg	2900	3000	3000	3000	3000	3025	3025	3025	3025
Crude protein	g/kg	216	208	198	188	178	198	188	178	168
Crude fat	g/kg	67	83	76	70	64	79	71	66	60
Crude fibre	g/kg	35	37	36	35	34	35	35	34	34
Crude ash	g/kg	54	48	47	46	44	43	42	40	39
Starch Brunt	g/kg	353	351	373	397	421	376	399	421	445
Calcium	g/kg	8.0	6.5	6.5	6.5	6.5	5.5	5.5	5.5	5.5
Phosphorus	g/kg	6.2	5.5	5.5	5.5	5.5	4.2	4.2	4.2	4.1
Sodium	g/kg	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Potassium	g/kg	9.7	9.3	8.7	8.0	7.2	8.7	8.2	7.5	6.7
Chloride	g/kg	1.5	1.5	1.5	1.5	1.4	1.5	1.5	1.5	1.5
dEB	meq	260	245	232	213	196	236	223	203	185
6-Phytase E4a1640	ftu	500	500	500	500	500	500	500	500	500
Dig.lysine	g/kg	11.5	10.5	10.5	10.5	10.5	9.9	9.9	9.9	9.9
Dig.methionine	g/kg	5.6	5.0	5.1	5.3	5.5	4.7	4.9	5.1	5.2
Dig.met+cys	g/kg	8.5	7.9	7.9	7.9	7.9	7.5	7.5	7.5	7.5
Dig.threonine	g/kg	7.5	6.8	6.8	6.8	6.8	6.4	6.4	6.4	6.4
Dig.tryptophan	g/kg	2.37	2.30	2.30	2.30	2.30	2.17	2.17	2.17	2.17
Dig.isoleucine	g/kg	7.8	7.4	7.4	7.4	7.4	7.0	7.0	7.0	7.0
Dig.valine	g/kg	9.2	8.4	8.4	8.4	8.4	7.9	7.9	7.9	7.9
Dig.arginine	g/kg	12.5	12.0	12.0	12.0	12.0	11.3	11.3	11.3	11.3
Dig.glycine + serine	g/kg	16.3	15.7	15.7	15.7	15.7	14.9	14.9	14.9	14.9
Dig. P broiler	g/kg	4.4	3.8	3.8	3.9	3.9	2.8	2.8	2.8	2.8

Appendix 3 Amino acid ratios to lysine

	Starter diet		Grower CP 205		Grower CP 195		Grower CP 185		Grower CP 175	
	g/kg	ratio lysine	g/kg	ratio lysine	g/kg	ratio lysine	g/kg	ratio lysine	g/kg	ratio lysine
Lysine	11.5	100%	10.5	100%	10.5	100%	10.5	100%	10.5	100%
Methionine	5.6	49%	5	48%	5.2	50%	5.3	50%	5.5	52%
Methionine+ cysteine	8.5	74%	7.9	75%	7.9	75%	7.9	75%	7.9	75%
Threonine	7.5	65%	6.8	65%	6.8	65%	6.8	65%	6.8	65%
Tryptophan	2.4	21%	2.2	21%	2.1	20%	1.9	18%	1.9	18%
Arginine	12.5	109%	11.8	112%	11.5	110%	11.6	110%	11.6	110%
Valine	8.5	74%	8.4	80%	8.4	80%	8.4	80%	8.4	80%
Isoleucine	7.8	68%	7.4	70%	7.3	70%	7.4	70%	7.4	70%
Glycine+serine	16.3	142%	15.6	149%	14.7	140%	14.7	140%	14.7	140%

	Finisher CP 195		Finisher CP 185		Finisher CP 175		Finisher CP 165	
	g/kg	ratio lysine	g/kg	ratio lysine	g/kg	ratio lysine	g/kg	ratio lysine
Lysine	9.9	100%	9.9	100%	9.9	100%	9.9	100%
Methionine	4.8	48%	4.9	49%	5.1	52%	5.3	54%
Methionine+ cysteine	7.5	76%	7.5	76%	7.5	76%	7.5	76%
Threonine	6.4	65%	6.4	65%	6.4	65%	6.4	65%
Tryptophan	2.1	21%	2.0	20%	1.8	18%	1.8	18%
Arginine	11.1	112%	10.9	110%	10.9	110%	10.9	110%
Valine	7.9	80%	7.9	80%	7.9	80%	7.9	80%
Isoleucine	6.9	70%	6.9	70%	6.9	70%	6.9	70%
Glycine+serine	14.8	149%	13.8	139%	13.7	138%	13.7	138%

Appendix 4 Overview of scores for foot-pad lesions

Scorekaart voetzoollaesies vleeskuikens (versie 1.2)

Klasse 0 - glad, geen laesie
Class 0 – smooth, no lesion

Klasse 0 – kleine verkleuring
Class 0 – small discolouration

Klasse 0 – bijna genezen laesie (litteken)
Class 0 – almost healed lesion, scar

Klasse 1 – oppervlakkige laesie, verkleuring
Class 1 – superficial lesion, discolouration

Klasse 1 – donkere papillen, geen ontsteking
Class 1 – dark papillae, no ulceration

Klasse 1 – aanzienlijke verkleuring
Class 1 – substantial discolouration

Klasse 2 – donkere papillen en ontsteking
Class 2 – dark papillae and ulcer

Klasse 2 – ontsteking bedekt met korst
Class 2 – ulcer covered by crust

Klasse 2 – ontsteking/bumble foot, gezwollen
Class 2 – abcess/bumble foot swollen

Appendix 5 Relative differences between calculated and analysed amino acid contents of the grower and finisher diets

Table A5.1 *Relative differences (in %) between calculated and analysed amino acids contents of the grower and finisher diets*

	Grower diets				Finisher diets			
	Control CP	**CP-1%**	**CP-2%**	**CP-3%**	**Control CP**	**CP-1%**	**CP-2%**	**CP-3%**
LYS	-2.5	-0.9	-0.9	-1.7	-3.6	-2.7	-3.6	-0.9
METH	-5.6	-5.5	-3.6	-5.2	-3.9	-3.8	-5.6	-3.6
CYS	-5.7	-5.9	-3.1	0.0	-2.9	-3.0	-3.2	0.0
THR	-1.2	1.3	-1.3	-1.3	-2.6	-2.6	-2.7	-1.4
TRP								
ILE	2.4	2.4	2.4	-1.2	1.3	2.6	0.0	1.3
ARG	0.0	3.1	2.4	0.8	-0.8	0.8	0.0	0.8
FEN	2.0	4.3	3.6	2.6	1.1	3.4	2.5	2.7
HIS	-3.8	0.0	-2.2	-2.4	-6.0	-2.1	-2.3	-5.0
LEU	-0.6	2.7	2.2	0.8	-3.3	2.1	0.0	2.4
TYR								
VAL	2.1	2.1	-1.0	-2.1	2.2	0.0	-1.1	-1.1
ALA	-0.2	3.0	2.6	2.1	-3.5	1.8	0.8	3.3
ASP	-0.2	2.1	0.4	-0.5	-2.1	1.6	1.0	-1.6
GLU	-0.2	-0.3	-2.0	-2.5	-1.1	-1.8	-3.3	-2.4
GLY	0.6	10.2	9.1	7.2	-2.0	10.6	11.0	11.8
PRO	-0.2	0.7	-1.1	0.2	-1.6	-2.1	-2.4	0.0
SER	-0.4	1.6	-0.5	2.4	-1.4	0.6	0.4	1.5

Appendix 6 Performance results 0 -11 days

Table A6.1 *Performance results starter phase (0 - 11 days)*

	Control CP	CP-1%	CP-2%	CP-3%	F-prob	LSD
Live weight (g)	324	323	323	321	0.656	4.31
Growth (g)	287	285	285	284	0.656	4.31
Growth (g/d)	26.1	26.0	25.9	25.8	0.656	0.39
Mortality (%)	0.0	0.9	0.0	1.4	0.401	1.93
FCR	1.101	1.106	1.106	1.107	0.851	0.0158
CP conversion	0.2378	0.2388	0.2389	0.2402	0.629	0.00364
Feed intake (g)	28.7	28.7	28.7	28.7		
Water intake (ml)	57.3	57.3	57.3	57.3		

Appendix 7 Effect diet composition on carbon footprint diet

		Diet composition									CFP	CFP per ingredient (g/CO₂-eq/kg)								
		Starter	Control CP	CP-1%	CP-2%	CP-3%	Control CP	CP-1%	CP-2%	CP-3%	gCO₂-eq/kg	Starter	Control CP	CP-1%	CP-2%	CP-3%	Control CP	CP-1%	CP-2%	CP-3%
Wheat	%	30.87	37.29	30.00	30.00	30.00	39.64	30.00	30.00	30.29	399	123	149	120	120	120	158	120	120	121
Corn	%	25.00	18.80	29.04	32.75	36.51	20.56	33.02	36.49	40.00	604	151	114	175	198	221	124	199	220	242
Soybean meal HiPro	%	30.47	27.34	24.84	21.07	17.30	24.99	22.55	18.41	14.62	649	198	177	161	137	112	162	146	119	95
Rapeseed meal 00	%	3.00	5.00	4.96	5.00	5.00	4.28	4.40	5.00	5.00	481	14	24	24	24	24	21	21	24	24
Palm oil crude	%	1.54	2.71	2.45	2.19	1.51	2.51	2.25	1.68	1.01	1591	25	43	39	35	24	40	36	27	16
Soya lecithin	%	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1591	16	16	16	16	16	16	16	16	16
Soya oil	%	0.92	1.51	0.87	0.50	0.50	1.22	0.50	0.50	0.50	1591	15	24	14	8	8	19	8	8	8
Lauric fatty acids	%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1591	8	8	8	8	8	8	8	8	8
Limestone	%	0.25	--	--	--	--	0.25	0.25	0.25	0.26	19	0	0	0	0	0	0	0	0	0
L-Lysine HCl	%	0.22	0.16	0.24	0.36	0.48	0.16	0.24	0.36	0.47	6030	13	9	14	21	29	10	14	22	29
Sodiumbicarbonate	%	0.28	0.21	0.25	0.31	0.39	0.24	0.28	0.34	0.41	1050	3	2	3	3	4	3	3	4	4
DL-methionine	%	0.28	0.23	0.25	0.29	0.32	0.21	0.24	0.27	0.31	5490	15	12	14	16	18	12	13	15	17
Sodium chloride	%	0.11	0.13	0.10	0.06		0.12	0.09	0.05		180	0	0	0	0	0	0	0	0	0
L-threonine	%	0.09	0.05	0.09	0.14	0.19	0.05	0.08	0.14	0.19	16978	15	8	15	24	33	8	14	23	32
L-Valine 98	%	0.07	0.02	0.07	0.14	0.21	0.02	0.07	0.14	0.20	9500	7	2	7	13	19	2	6	13	19
Xylanase 6,25%	%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	4999	5	5	5	5	5	5	5	5	5
Phyzyme XP 5000L	%	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	4999	0	0	0	0	0	0	0	0	0
Oat hulls	%	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	478	10	10	10	10	10	10	10	10	10
Monocalciumphosphate	%	0.99	0.66	0.69	0.76	0.82	0.15	0.19	0.22	0.26	4999	49	33	35	38	41	7	9	11	13
L-Arginine	%	--	--	0.08	0.19	0.30	--	0.08	0.19	0.30	9500	0	0	8	18	28	0	8	18	28
L-Isoleucine	%	--	--	0.04	0.11	0.18	--	0.04	0.11	0.18	9500	0	0	4	10	17	0	4	11	17
L-Glycine	%	--	--	0.09	0.21	0.34	--	0.09	0.22	0.34	9500	0	0	8	20	32	0	8	20	32
L-Tryptophan	%	--	--	0.02	0.04	0.06	--	0.02	0.04	0.06	9500	0	0	2	4	6	0	2	4	6
VM VLK 20-40	%	2.00	2.00	2.00	2.00	2.00	--	--	--	--	4999	100	100	100	100	100	0	0	0	40
VM VLK 0-20 dgn	%	--	--	--	--	--	2.00	2.00	2.00	2.00	4999	0	0	0	0	0	100	100	100	100
VM Maxiban 0,3%	%	0.30	0.30	0.30	0.30	0.30					4999	15	15	15	15	15	0	0	0	0
										Total		783	752	796	842	888	704	752	797	841

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the potential
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