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

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ABSTRACT A study with 884 Ross 308 male broilers, housed in 68 floor pens (0.75 m^2) from 0 to 35 days of age was conducted to evaluate the effects of low crude protein (CP) diets, with partial replacement of soybean meal by free amino acids (AA), on performance, slaughter yields, litter quality and footpad lesions. During the first 11 d, all broilers received the same control starter diet (216 g/kg CP, 11.5 g/kg apparent fecal digestible (AFD) lysine, and 2900 kcal/kg AMEn). Thereafter, four experimental feeding programs with different levels of dietary CP (control and control with 1% (CP-1%), 2% (CP-2%) and 3% (CP-3%) less CP units) were provided in both the grower and finisher phase. In the control grower and finisher diet, the CP content was 208 and 198 g/kg, respectively. All diets were formulated to meet or exceed the recommendations concerning AFD AA, and to be iso-caloric within each feeding phase. Feed and water were provided for *ad libitum* intake during the entire experimental period.

None of the low CP feeding programs affected body weight gain, feed intake or mortality from 0 to 35 d. However, CP conversion was improved with the reduction of CP content of the diet. Broilers fed the CP-2% or CP-3% feeding program had an improved feed conversion ratio. Broilers fed the low CP protein feeding programs had a better litter quality and less footpad lesions, compared to broilers fed the control feeding program. Broilers fed the CP-3% feeding program had a lower breast meat yield than broilers fed the control feeding program. Slaughter yields of broilers fed CP-1% or CP-2% feeding program did not differ from the control feeding program. This study demonstrated that the CP content of grower and finisher diets can be reduced by 2.2–2.3% units without adverse effects on growth performance of broilers, while CP reduction seems promising to reduce nitrogen excretion from broiler houses, improve bird welfare, and reduces dependence on vegetable protein sources.

Key words: Broiler, low protein, growth performance, litter quality, footpad lesion

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INTRODUCTION

World population is expected to grow from 7.7 billion in 2018 to almost 10 billion in 2050. This trend and the expected increase in prosperity, implies that the market demand for animal derived food will continue to grow (FAO, 2009; FAO, 2017). It is expected that the demand for poultry meat will increase rapidly worldwide. As a result, the demand for vegetable proteins, e.g., soybean meal, for use in animal feed will increase (Alexandratos and Bruinsma, 2012). In Europe, the rate of self-sufficiency for soybean meal is only 5% (EU, 2017). This strong dependency for this important protein source in poultry diets on other areas, mainly South America makes the EU livestock sector vulnerable to price volatility and trade distortions, causing feed price to rise, thereby, increasing farmers' production costs and reducing the sectors' profitability (Euractiv, 2011). On the other hand, there is concern about the deforestation of tropical rain forest which is done to fulfil the need for arable land for soybean cultivation (WNF, 2011; Van Gelder and Kuepper, 2012).

Reduction of the CP of broiler diets can contribute to reduce the import and use of soybeans in Europe as well. However, reducing the CP content of broiler diets will pose a risk for reduced growth performance, when (semi-) essential AA become limiting. When the CP content is reduced, glycine and serine levels among other nonessential AA decrease as well (Dean et al., 2006). Therefore, it is possible that marginal levels of dietary glycine and serine cause a decrease in performance of broilers when feeding low CP diets, even

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if these are supplemented with essential AA up to bird's requirement (e.g., Ferguson et al., 1998a, b; Bregendahl et al., 2002; Veldkamp et al., 2017). Several studies showed that glycine supplementation prevented the adverse effects of low CP diets on broiler performance (Dean et al. 2006; Ospina-Rojas et al., 2012; Ospina-Rojas et al., 2013; Ospina Rojas et al., 2014).

A large part of the dietary nitrogen intake is not retained by the animal, but excreted into the environment. Lowering the CP content of the diet could therefore be a tool to reduce the nitrogen excretion and ammonia emission from broiler houses (Elwinger and Svensson, 1996; Kidd et al., 1996; Van Harn and Van Middelkoop, 1996; Ferguson et al., 1998a; Nahm, 2002; Khajali and Moghaddam, 2006; Namroud, et al., 2008; Hernandez et al., 2013). A reduced CP content in broiler diets can also reduce water intake, because there is a reduced need to excrete the surplus of nitrogen (Elwinger and Svensson, 1996; Alleman and Leclercq, 1997; Bailey, 1999). A lower water intake might reduce the risk of wet litter, and thus the risk of impaired welfare, since wet litter is the main reason for skin dermatitis such as footpad lesions, hock burns and breast blisters (Martland, 1985). Wet litter could also lead to more rejections at the slaughter house (Shepherd and Fairchild, 2010), because there is a high probability of broilers in permanent contact with wet and sticky litter developing contact dermatitis caused by humidity (water), non-identified irritants in the fecal materials and by ammonia (Allain et al., 2009). Moreover, field experiences show that low CP levels in broiler diets can reduce the risk of digestion problems and necrotic enteritis.

In summary, reduction of the dietary protein content has a number of potential benefits on EU protein self-sufficiency, environmental pollution and broiler health and welfare, provided that the growth performance and slaughter yield are not compromised. The objective of this study was to evaluate the effects of a reduction of 1, 2 or 3% units in dietary CP content in the grower (11–28 d) and finisher phase (28– 35 days), with a partial replacement of soybean meal by free AA (including glycine), on growth performance, slaughter yields, litter quality and footpad lesions of broilers.

MATERIALS AND METHODS

This experiment was performed in accordance with the Dutch rules and regulations and approved by the Ethics Committee of Wageningen University and Research, the Netherlands.

Diets and Diet Analysis

The experimental diets were formulated and produced by ForFarmers, Heijen, The Netherlands. A three-phase feeding program was applied. Starter, grower and finisher diets were provided from 0 to 11, 11 to 28 and 28 to 35 days of age, respectively. During the starter phase, all animals received the same control diet with a CP content of 216 g/kg and 11.5 g/kg digestible Lys. Thereafter, four experimental feeding programs differing in CP levels (control and with 1% (CP-1%), 2% (CP-2%) and 3% (CP-3%) lower dietary CP content in each phase) were provided. The control diets had a CP content in the grower and finisher phase of 208 and 198 g/kg, respectively. The diets were formulated to meet or exceed the recommendations for apparent fecal digestible AA (lysine, methionine, threonine, tryptophan, isoleucine, valine, arginine) according to CVB recommendations (CVB, 2012), and to be iso-energetic within each phase. Because of the importance of glycine + serine in low protein diets, it was decided in the present study to add free glycine to the low protein diets to the apparent fecal digestible glycine + serine in the control diet, to maintain the production results of broilers. Free glycine was not approved to be used as a feed additive in The Netherlands. Therefore, permission (BD 15.290/FV/MS) from the Veterinary Medicinal Products Unit was obtained to carry out this study. All diets of each phase were prepared with the same batch of ingredients. 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 ingredient and calculated nutrient compositions of the diets are given in Table 1.

All diets were pelleted (starter diets as 2.5 mm diameter pellets, grower and finisher diets both as 3.2 mm diameter pellets). All diets were analyzed for dry matter, ash, crude protein, crude fat, crude fiber, starch and AA (except tryptophan and tyrosine). 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.

Dry matter content was determined gravimetrically after 4 h at 103°C (ISO, 1998). The content of ash was determined gravimetrically after ashing at 550°C (ISO, 2002). Nitrogen content was determined by the Kjeldahl method (ISO, 1997). Crude fat was performed with a gravimetrical method based on EC 3–9-1998; nr. L 257/23–25. For crude fiber a method with intermediate filtration was used (ISO, 2000). Starch content was determined enzymatically according to NEN-EN-ISO 15914 (2005). The AA were assayed by ion exchange chromatography in an Amino Acid Analyzer (Evonik, 2010).

Birds and Management

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 each pen, 13 broilers

Table 1. Ingredient and calculated nutritional composition of the starter (0 to 11 d), grower (11 to 28 d) and finisher diets (28 to 35 d) to determine the effect of a reduction of 1 to 3%-units of crude protein (CP) in grower and finisher diets.

			Grower diets				Finisher diets			
		Starter diet	Control CP	CP-1%	CP-2%	CP-3%	Control CP	CP-1%	CP-2%	CP-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 48%	%	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
Oat hulls	%	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Palm oil	%	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
Mono calcium phosphate	%	0.99	0.66	0.69	0.76	0.82	0.15	0.19	0.22	0.26
Limestone	%	0.25	–	–	–	–	0.25	0.25	0.25	0.26
Sodium bicarbonate	%	0.28	0.21	0.25	0.31	0.39	0.24	0.28	0.34	0.41
Sodium chloride	%	0.11	0.13	0.10	0.06	–	0.12	0.09	0.05	–
L-Lysine HCl	%	0.22	0.16	0.24	0.36	0.48	0.16	0.24	0.36	0.47
DL-Methionine	%	0.28	0.23	0.25	0.29	0.32	0.21	0.24	0.27	0.31
L-Threonine	%	0.09	0.05	0.09	0.14	0.19	0.05	0.08	0.14	0.19
L-Valine	%	0.07	0.02	0.07	0.14	0.21	0.02	0.07	0.14	0.20
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
Glycine	%	–	–	0.09	0.21	0.34	–	0.09	0.22	0.34
L-Tryptophan	%	–	–	0.02	0.04	0.06	–	0.02	0.04	0.06
Premix A[1]	%	2.00	2.00	2.00	2.00	2.00	–	–	–	–
Premix B[2]	%	–	–	–	–	–	2.00	2.00	2.00	2.00
Maxiban G160 (anti-coccidial)[3]	%	0.30	0.30	0.30	0.30	0.30	–	–	–	–
Xylanase 6.25% (Endo-1,4-beta-xylanase)[4]	%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phyzyme® XP 5000 L (phytase enzyme)[5]	%	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition										
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 fiber	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	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[6]	meq	260	245	232	213	196	236	223	203	185
6-Phytase E4a1640	FYT/kg	500	500	500	500	500	500	500	500	500
AFD[7] lysine	g/kg	11.5	10.5	10.5	10.5	10.5	9.9	9.9	9.9	9.9
AFD methionine	g/kg	5.6	5.0	5.1	5.3	5.5	4.7	4.9	5.1	5.2
AFD Met+Cys	g/kg	8.5	7.9	7.9	7.9	7.9	7.5	7.5	7.5	7.5
AFD threonine	g/kg	7.5	6.8	6.8	6.8	6.8	6.4	6.4	6.4	6.4
AFD tryptophan	g/kg	2.37	2.30	2.30	2.30	2.30	2.17	2.17	2.17	2.17
AFD isoleucine	g/kg	7.8	7.4	7.4	7.4	7.4	7.0	7.0	7.0	7.0
AFD valine	g/kg	9.2	8.4	8.4	8.4	8.4	7.9	7.9	7.9	7.9
AFD arginine	g/kg	12.5	12.0	12.0	12.0	12.0	11.3	11.3	11.3	11.3
AFD glycine + serine	g/kg	16.3	15.7	15.7	15.7	15.7	14.9	14.9	14.9	14.9
AFD P broiler	g/kg	4.4	3.8	3.8	3.9	3.9	2.8	2.8	2.8	2.8

[1]Premix provided per kg of diet: 10,000 IU vitamin A (retinylacetate), 3500 IU vitamin D3 (cholecalciferol), 0.025 mg 25-hydroxycholecalciferol, 100 IU vitamin E (all-rac alpha tocopheryl acetate), 4 mg vitamin K3 (menadione nicotinamide bisulfite), 4 mg vitamin B1 (thiamine mononitrate), 9 mg vitamin B2 (riboflavin), 21.7 mg d-pantothenic acid, 70 mg niacin amide, 250 μg biotin, 30 μg vitamin B12 (cyanocobalamin), 1.5 mg folic acid, 6 mg vitamin B6 (pyridoxine hydrochloride), 150 mg betaine, 30 mg L-carnitine, 50 mg Fe (as FeSO4•H2O), 10 mg Cu (as $CuSO_4$•5H2O), 4 mg Cu (as Cu chelate), 70 mg Zn (as ZnSO4•H2O), 30 mg Zn (as Zn chelate), 40 mg Mn (as MnSO4•H2O), 15 mg Mn (as Mn chelate), 2.0 mg I (as CaI2), 0.1 mg Se (as $Na2SeO_3$•$5H_2O$), 0.2 mg selenomethionine, 2 mg E310 (propyl gallate), 1 mg E320 (butylated hydroxyanisole, BHA) and 1.3 mg E321 (butylated hydroxytoluene, BHT).

[2]Premix provided per kg of diet: 10,000 IU vitamin A (retinylacetate), 3000 IU vitamin D3 (cholecalciferol), 45 IU vitamin E (all-rac alpha tocopheryl acetate), 3 mg vitamin K3 (menadione nicotinamide bisulfite), 2 mg vitamin B1 (thiamine mononitrate), 7 mg vitamin B2 (riboflavin), 16.3 mg d-pantothenic acid, 60 mg niacin amide, 200 μg biotin, 25 μg vitamin B12 (cyanocobalamin), 1 mg folic acid, 5 mg vitamin B6 (pyridoxine hydrochloride), 100 mg betaine, 30 mg L-carnitine, 30 mg Fe (as FeSO4•H2O), 10 mg Cu (as CuSO4•5H2O), 80 mg Zn (as ZnSO4•H2O), 20 mg Zn (as Zn chelate), 40 mg Mn (as MnSO4•H2O), 2.0 mg I (as CaI2), 0.1 mg Se (as Na2SeO3•5H2O), 0.1 mg selenomethionine, 2 mg E310 (propyl gallate), 1 mg E320 (butylated hydroxyanisole, BHA) and 1 mg E321 (butylated hydroxytoluene, BHT).

[3]Maxiban G160, Trouw Nutrition Nederland B.V., The Netherlands

[4]Xylanase 6.25% (EC Registration Number: 4a11, EC 3.2.1.8), Trouw Nutrition Nederland B.V., The Netherlands

[5]Phyzyme® XP 5000 L (EC Registration Number: 4a1640, EC 3.1.3.26), Danisco, Malborough, United Kingdom

[6]dEB = dietary electrolyte balance (= Na++K+-Cl−)

[7]AFD = Apparent Fecal Digestibility CVB Feed Table (2007)

Table 2. Description of scoreyes for friability and wetness of the litter.

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

were placed. The floor pens were located in a naturally ventilated poultry house. Sixty-eight of these 72 pens were used for this trial. In the remaining four pens, spare animals were housed, which were used to maintain the number of animals in the experimental pens in case of mortality during the starter phase (0 to 11 d).

The birds were visually observed twice a day to check animal health. All broilers were vaccinated against New Castle Disease (Nobilis® ND Clone 30, MSD Animal Health, spray vaccination) at 15 days of age at the experimental facility. Water and feed were provided *ad libitum* during the entire experimental period (0–35 days of age). Feed was supplied via feeding bins (0.75 m feeding space/pen). Water was supplied by one drinking cup (Impex, Barneveld, the Netherlands) per pen. The temperature at placement of the broilers was 34°C and it was gradually decreased to 20°C at 34 days of age. During the first two days light was nearly continuously switched on (23L:1D), from 3–35 days of age a day/night schedule of 18 h light and 6 h dark (18L:6D) was given per 24 h. Light intensity was 20 lux at bird level during the entire experimental period.

Growth Performance

Body weight (BW) of the birds was determined per pen at 0, 11, 28 and 35 days of age. Feed intake per pen was determined at 11, 28 and 35 days of age as provided feed minus remaining feed in each feeding phase. Body weight gain (BWG, g), average daily gain (ADG, g/d), feed conversion ratio (FCR, g/g), feed intake (FI, g), and average daily feed intake (ADFI, g/d) 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 × BWG; ADFI = FI/length period. The crude protein conversion (CPC) was calculated over the above mentioned periods as CPC = FI (kg) × CP content diet (g/kg)/BWG (g). Culling, mortality and health (including probable causes of any culling, illness or deaths) were recorded daily. EPEF (European Production Efficiency Factor) was calculated per pen at 35 days as (mean daily body weight gain (g) × (100–% mortality)/(FCR × 10).

Slaughter Yields

Slaughter yields of 10 randomly selected birds per pen were determined at 35 days of age. Selected birds were removed, individually marked, weighed and transported to a commercial slaughter house. At the slaughter house, the broilers were manually dissected by trained personnel to determine carcass, wing, leg (thigh and drums), back and breast meat 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 and Litter Composition

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.

At the end of the study, representative samples of the litter were taken from all pens. These samples were analyzed for dry matter, total N, ammoniacal N and pH. These analyses were performed by the service lab of Wageningen Livestock Research, Wageningen, the Netherlands. Dry matter and total N were measured according to NEN 7432 (1998) and NEN 7434 (1998), respectively. Ammoniacal N was determined according to NEN 7438 (1997). For the pH determination, 50 grams of litter material was added to 200 mL demi-water, thoroughly mixed and after 16 hours the pH was measured with a pH electrode (XS-instruments pH 8).

Table 3. Calculated and analyzed nutrient composition (g/kg) of the grower and finisher diets fed to broilers to determine the effect of a reduction of 1 to 3%-units of crude protein (CP).

Nutrient		Grower diets				Finisher diets			
		Control CP	CP-1%	CP-2%	CP-3%	Control CP	CP-1%	CP-2%	CP-3%
Calculated									
Crude protein	g/kg	208	198	188	178	198	188	178	168
Crude fat	g/kg	83	76	70	64	79	71	66	60
Crude fiber	g/kg	37	36	35	34	35	35	34	34
Crude ash	g/kg	48	47	46	44	43	42	40	39
Starch	g/kg	351	373	397	421	376	399	421	445
Analyzed									
Crude protein	g/kg	200	194	186	178	191	184	176	168
Crude fat	g/kg	81	76	70	66	75	71	66	61
Crude fiber	g/kg	37	37	35	36	37	38	38	34
Crude ash	g/kg	47	46	45	42	42	40	39	36
Starch	g/kg	350	364	385	404	359	379	407	432

Footpad Lesions

Occurrence and severity of footpad lesions (scale 0, 1, 2) was determined at 34 days of age by an experienced assessor. All broilers in a pen were used for these assessments. Footpad lesions were scored per broiler for both feet according to Berg (1998), i.e., score 0: no lesions or very small discoloration; score 1: discoloration but no deep lesion; score 2: deep lesion with ulcers or scabs, bumble foot. The severity of footpad lesions was expressed as footpad score (FPS) per pen. This score was calculated as: 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 FPS can range from 0 (all birds having no lesions) to 200 (all birds having score 2).

Statistical Analysis

Raw data were analyzed for statistical outliers. An outlier was defined as an observation deviating more than 2.5 standard deviations from the mean. No outliers, however, were detected, meaning that all data were included in the statistical analysis. Pen was served as the experimental unit and statistical analyses were carried out using Genstat (18th edition, VSN International LTD, Hemel Hempstead, UK). All data were analyzed using ANOVA with groups of four consecutive pens included as a blocking factor and diet as fixed factor (explanatory variable). Differences between treatments were analyzed using Fisher's Least Significant Difference (LSD) in case the treatment effect was significant ($P \leq 0.05$).

RESULTS

Diet Composition

The analyzed nutrient contents of the experimental diets were consistent with the expected values (Table 3), except for the CP content of the control grower and finisher diets, which were 8 and 7 g/kg lower than calculated values, respectively. The absolute difference in analyzed crude protein content between control and CP-3% was 2.2% and 2.3% for the grower and finisher diet, respectively, instead of the calculated difference of 3%. In general, the analyzed AA contents met the calculated contents, except for the glycine level of the glycine supplemented diets which was on average 10% higher than calculated (Table 4).

Growth Performance

In the grower phase (12–28 d), finisher phase (29–35 d) and over the entire experimental period (0–35 d), no adverse effects of the reduction in dietary CP content on BW, BWG, mortality and FI were observed (Table 5). In the finisher phase ($P<0.01$) and in the entire period ($P = 0.002$), the reduction in dietary CP content decreased the FCR of the birds with the lowest FCR in birds of the 2 and 3% reduced CP content. In all feeding phases and overall, the protein efficiency increased with each reduction of the dietary CP content as indicated by the decrease ($P<0.001$) of the CP conversion (CP intake per g of body gain, CPC). Broilers fed the CP-2% feeding program had the highest ($P = 0.035$) EPEF. The EPEF of the broilers receiving the CP-1% or CP-3% feeding program did not differ from the control.

Broilers fed the control feeding program had the highest incidence and the most severe footpad lesions as indicated by the highest FPS ($P < .001$). The footpad score decreased with each reduction of the CP content of the diet.

Slaughter Yields

In Table 6, the slaughter yields per treatment on 35 days of age are given. Providing broilers with low CP diets had no significant effect on live weight, carcass weight, and carcass yield. Relative to the carcass weight, broilers fed the CP-3% diets program had a lower wing and breast meat yield ($P < .001$), but a higher leg ($P < 0.028$) and back yield ($P < 0.028$) compared to the control group. Absolute weights of the

Table 4. Calculated and analyzed amino acid contents of the grower and finisher diets fed to broilers to determine the effect of a reduction of 1 to 3%-units of crude protein (CP).

		Grower diets				Finisher diets			
		Control CP (= 208 g/kg CP)	CP-1%	CP-2%	CP-3%	Control CP (= 198 g/kg CP)	CP-1%	CP-2%	CP-3%pt
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
Valine	g/kg	9.7	9.7	9.6	9.5	9.2	9.2	9.1	9.0
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
Alanine	g/kg	9.1	8.7	8.1	7.6	8.8	8.4	7.8	7.2
Aspartic acid	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
Proline	g/kg	12.6	12.2	11.5	10.8	12.3	11.8	11.1	10.3
Glycine	g/kg	8.6	8.2	8.7	9.2	8.2	7.7	8.1	8.5
Serine	g/kg	9.8	9.2	8.5	7.8	9.3	8.7	7.9	7.3
Gly+Ser	g/kg	18.4	17.4	17.2	17.7	17.5	16.4	16.0	15.8
Analyzed									
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
Valine	g/kg	9.9	9.9	9.5	9.3	9.4	9.2	9.0	8.9
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							
Alanine	g/kg	9.1	9.0	8.3	7.8	8.5	8.6	7.9	7.4
Aspartic acid	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
Proline	g/kg	12.6	12.3	11.4	10.8	12.1	11.6	10.8	10.3
Glycine	g/kg	8.7	9.0	9.5	9.9	8.0	8.5	9.0	9.5
Serine	g/kg	9.8	9.4	8.5	8.0	9.2	8.8	7.9	7.4
Gly+Ser	g/kg	18.4	18.4	18.0	17.9	17.2	17.3	16.9	16.9

wing, back and breast meat did not differ between the control group and the CP-3% group. Broilers fed the CP-3% feeding program had a higher leg weight compared to broilers that received the control feeding program ($P < 0.035$). No differences in slaughter yields were found between broilers fed the control, CP-1% or CP-2% diets.

Litter Quality Assessment and Litter Composition

Table 7 presents the visual litter quality (friability and wetness) and the litter composition at 35 days of age. Visual litter quality linearly improved, indicating more friable and dryer litter, with decreasing CP content of the diet ($P < .001$). The results of the visual litter quality corresponded reasonably well with the dry matter (DM) contents of the litter. Dry matter content of the litter of broilers fed with the CP-3% feeding program was higher compared with the litter DM-content of the control fed broilers, where the litter DM-contents of the CP-1% and CP-2% groups were in between ($P < 0.033$).

Total nitrogen (N) of the litter of the control and the CP-1% fed birds was significantly higher than the litter total-N of the CP-3% fed group ($P < 0.002$). The litter total-N of the CP-2% fed animals was in between and did not differ from either the control or the CP-3% group. Litter ammoniacal N content of broilers fed the CP-3% was significantly lower than of broilers fed the CP-1% diets, but it did not significantly differ from the control and CP-2% fed groups ($P < 0.026$). Dietary treatment had no effect on the pH of the litter.

DISCUSSION

This study demonstrated that growth performance of broilers fed low protein diets was unaffected or increased when decreasing dietary CP in the grower (11 to 28 d) and finisher phase (28 to 35 d) with 1 to 3% units (10 to 30 grams per kg of diet), when such diets were supplemented with essential AA including glycine

Table 5. Influence of a 1 to 3%-units crude protein (CP) reduction in grower and finisher diets on growth performance of male broilers from 11–28 d (grower phase), 28–35 d (finisher phase) and 0–35 d of age (entire experimental period).

	Control CP	CP-1%	CP-2%	CP-3%	P-value	LSD
Calculated CP content grower phase (in g/kg)	208	198	188	178		
Calculated CP content finisher phase (in g/kg)	198	188	178	168		
11–28 days						
Body weight d11	324	323	323	321	0.656	4.3
Body weight d28 (g)	1752	1751	1752	1739	0.759	29.1
Body weight gain (g/d)	84.0	84.0	84.1	83.4	0.811	1.63
Mortality (%)	3.2	3.6	2.0	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 (CPC)[1]	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
28–35 days						
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 (CPC)[1]	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
0–35 days						
Body weight d35 (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 (g/g)	1.549[a]	1.542[ab]	1.505[c]	1.510[bc]	0.002	0.0265
Crude protein conversion (CPC)[1]	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
European Production Efficiency Factor (EPEF) [2]	413[b]	417[b]	447[a]	428[ab]	0.035	24.5
Footpad score (FPS) [3]	143[a]	110[b]	79[c]	39[d]	<.001	28.1

[a–d]Values without a common superscript per row differ significantly ($P < 0.05$).
[1]CPC = FI (kg) × analyzed CP content diet (g/kg)/BWG (g)
[2]EPEF = (daily body weight gain (g) × (100–% mortality)/FCR × 10)
[3]FPS = 100% × ((0.5 × n birds with score 1) + (2 × n birds with score 2))/n total birds.

Table 6. Influence of a 1 to 3%-units crude protein (CP) reduction in grower and finisher diets on the slaughter yields of male broilers on 35 d of age. Slaughter yields were based on 10 randomly selected birds per pen.

	Control CP	CP-1%	CP-2%	CP-3%	F-prob.	LSD
Calculated CP content grower phase (in g/kg)	208	198	188	178		
Calculated CP content finisher phase (in g/kg)	198	188	178	168		
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 yield (% 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 (g)	520[ab]	532[b]	526[ab]	513[a]	0.046	13.5
Breast meat (% of CW)	32.1[ab]	32.3[a]	31.9[b]	31.2[c]	<.001	0.39

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

up to the same level of the control. Providing broilers with low CP diets, even when supplemented with free AA, often resulted in deteriorated growth performance (e.g., Ferguson et al., 1998a, b; Bregendahl et al., 2002). In the present study, no difference in BWG was found between the control fed birds and the broilers who received the AA supplemented low CP diets. Feed conversion ratio of the broilers receiving the CP-2% and CP-3% diets was improved compared to the control fed birds. It might be that the decreased growth performance results of broilers fed the control diet could be explained by the deteriorated litter quality (Greene et al., 1985; Martland, 1985). The deteriorated litter quality (more caked and wetter) 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 (De Jong et al., 2014).

Another reason for the deteriorated FCR of the control fed birds could be a poorer intestinal health. It is well known that high protein diets might have negative effects on gut health and performance (Qaisrani et al., 2015; Apajalahti and Vienola, 2016). Protein that was not digested up to the end of the small intestine can potentially be fermented by putrefactive bacteria in the caecum. Putrefaction produces many harmful and toxic compounds like amines, indoles, phenols, cresol and ammonia, which in high concentrations may have

Table 7. Influence of a 1 to 3%-units crude protein (CP) reduction in grower and finisher diets on the visual quality (friability and wetness) and dry matter, total nitrogen, ammoniacal nitrogen and pH content of the litter on 35 days of age.

	Control CP	CP-1%	CP-2%	CP-3%	F-prob.	LSD
Calculated CP content grower phase (in g/kg)	208	198	188	178		
Calculated CP content finisher phase (in g/kg)	198	188	178	168		
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
Ammoniacal 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

[a–c]Values without a common superscript per row differ significantly ($P < 0.05$).
[1]1–10: 1 completely caked–10: completely friable; [2]1 -10: 1 very wet–10 dry

adverse effects on chicken growth and performance (Apajalahti and Vienola, 2016). Reduction of the protein bypassing the small intestine, by reducing the dietary CP content, might reduce the production of toxic protein fermentation metabolites in the caeca.

Moreover, the inclusion of free glycine in the low CP diets in the present study, whereby the glycine and serine recommendation (CVB, 2018) of the animal is covered, could be a reason for the similar (CP-1% and CP-3% feeding program) or even better (CP-2% feeding program) growth performance results in the low protein groups. According to Ospina-Rojas et al. (2013), supplemental glycine may be necessary to support maximum performance for broiler chickens when they are fed diets based on vegetable ingredients and with low protein levels.

The incidence of footpad lesions in the present study decreased linearly with the reduction of the dietary CP content. It is well known that the most important factor causing footpad lesions is considered to be wet litter (Shepherd and Fairchild, 2010). In the present study, the observed differences in litter quality are in good agreement with the determined footpad scores. Broilers fed the control feeding program had the lowest scores for litter quality and the highest footpad scores. The share of soybean meal decreased with the decrease in the protein content of the diets. As a result, the potassium content and the electrolyte balance (dEB) also decreased with the protein content of the diets. It is well known that water intake increases with the increase of both dEB and potassium (Mushtaq et al., 2013) and that this is the most likely reason for the deteriorated litter quality and the higher footpad score of the broilers fed the control diet. In addition to potassium, soybean meal contains also other components that can be responsible for a higher water excretion, such as fiber with high water retention capacity and fermentable sugars (Francesch and Brufau, 2004).

Reducing dietary CP resulted in a decrease of the litter moisture and nitrogen content. These results were in agreement with the findings of Ferguson et al. (1998a), Kamran et al. (2010) and Belloir et al. (2017), 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 reported a decrease in the nitrogen content, but no change in the moisture content of the litter. Several studies have shown a reduction in nitrogen excretion by about 10% for each 1 percentage point reduction in the dietary CP content in broilers (Ferguson et al. (1998a); Aletor et al. 2000; Bregendahl et al., 2002). The measured reduction in total nitrogen in the present study, however, was only 3 to 4% per percentage point lower dietary CP. In this study, none of the low protein groups had a significantly lower ammoniacal N compared with the control group. This might be caused by the fact that the process of uric acid conversion to ammoniacal N is depending on a lot more factors than ammoniacal N content only. The conversion of uric acid is also influenced by temperature, pH, oxygen and moisture content (Groot Koerkamp, 1994).

In previous experiments, feeding low protein diets did not always affect litter moisture content (Elwinger and Svensson, 1996; Ferguson et al., 1998b; Hernandez et al., 2013; and Ospina-Rojas et al., 2014). Differences in season, diet composition, raw material composition, housing, animal health, equipment (e.g., drinking system) and management (in particular stocking density, ventilation and heating) could explain absence of effects on litter moisture content in the present study (Dunlop et al., 2016).

In the present study, no effects of the dietary CP content on the acidity or pH of the litter were observed, 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). In contrast to our study, Ferguson et al. (1998a) reported a decrease in the pH of the litter with diets with a reduction in dietary CP content. The pH of the litter is influenced by many factors that could cause these different findings, e.g., diet composition, type of bedding material, uric-acid conversion rate.

Broilers fed the CP-3% feeding program had a lower wing and breast meat yield but a higher leg and back yield compared to the control group. However, due to the higher BW at slaughter, the absolute weight of the different parts did not differ from the control fed broilers. Feeding AA supplemented diets with up to two percent lower CP 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 in diets with a 3% units reduction in crude protein AA supplemented diets. According to Ospina-Rojas et al. (2014), it is important that glycine and arginine are added to low protein diets, accompanied by the supplementation of valine and isoleucine, besides lysine, methionine and threonine, to maintain slaughter yields. In the present study, the contents of essential AA, including glycine, of the low protein diets met CVB recommendations (CVB, 2018). The results of this study regarding breast meat yield were in agreement with the study of Aletor et al. (2000), who observed no effects on slaughter yields when CP content of the diet was reduced from 225 to 153 g/kg. Belloir et al. (2017) found no effects on breast meat yield up to 3% point reduction of the CP content of the diet.

CONCLUSIONS

The present study in growing broilers showed that a 2.2–2.3% units (22–23 g/kg) reduction of the CP content of grower and finisher diets, with adequate supplementation of essential amino acids, including lysine, methionine, threonine, arginine, isoleucine, valine and glycine, did not result in adverse effects on growth performance and slaughter yields. Moreover, the CP reduction reduced nitrogen and moisture content of litter and occurrence and severity of footpad lesions. Thus, reducing dietary CP seems to be a promising approach to reduce nitrogen excretion from broiler houses and to reduce the amount of vegetable protein in broiler diets, while simultaneously improving broiler welfare.

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