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Original Research Article

Reduction of dietary crude protein and feed form: Impact on broiler litter quality, ammonia concentrations, excreta composition, performance, welfare, and meat quality

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ABSTRACT

Nitrogen (N) excreted by poultry is converted to ammonia (NH_3), presenting an environmental risk and a health risk to the farmer and animals. A study was performed to investigate the effect of reduced CP and feed form on broiler performance and welfare, meat and litter quality, N utilization, and NH_3 concentrations at litter level. A total of 2,232 Ross 308 male broilers was divided into 6 treatments and 6 replicates, which was fed diets in both pellet and mash forms with different CP levels of 205.0 g/kg (H, high), 187.5 g/kg (M, intermediate) and 175.0 g/kg (L, low) in the grower phase and 195.0 g/kg (H), 180.0 g/kg (M) and 165.6 g/kg (L) in the finisher phase. Individual amino acids (AA) were supplemented to maintain digestible AA-to-digestible lysine ratios. Decreasing dietary CP content to 187.5 g/kg in the grower phase and 180.0 g/kg in the finisher phase reduced NH_3 concentrations at litter level ($P < 0.001$), but a further reduction in dietary CP had no additional effect. Mash treatments had better litter qualities and lower incidences of foot and hock lesions than pellet treatments at d 38 ($P < 0.001$). In addition, treatments with reduced CP had lower incidence of foot lesions at d 38 ($P < 0.001$). Broilers fed pelleted diets had higher ADFI, ADG, and final BW, improved feed conversion ratio (FCR), and heavier carcasses ($P < 0.001$) than those fed mash diets over a production period of 39 d. Performance could not be maintained when birds were fed L CP pelleted diets. This study demonstrated that, with the supplementation of AA to meet requirements, the concentration of dietary CP can be reduced to 187.5 and 180.0 g/kg in the grower and finisher phases respectively, without impairing broiler performance, meat yield and quality. Mash diets were favorable when considering the overall litter quality and welfare of the birds. However, they could not maintain the same broiler performance and slaughter yield as pelleted diets. Results from the present study may assist the poultry sector towards a socially acceptable low-emission farming system.

1. Introduction

In birds, absorption of excess amino acids (AA) is mainly excreted as uric acid while undigested protein is excreted via the feces. Microbial degradation of uric acid and undigested protein in the litter produces ammonia (NH_3) as a byproduct. Factors influencing the formation of NH_3 include the amount of available substrate, moisture content, pH, and temperature (Groot Koerkamp, 1994). Excreted nitrogen (N) is also associated with deteriorating litter quality, which comprises an increase in litter moisture and NH_3 content and contributes to the prevalence of

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foot and hock lesions in broilers (Nagaraj et al., 2007; Shepherd and Fairchild, 2010; De Jong et al., 2014; Taira et al., 2014; Dunlop et al., 2016). High NH_3 concentrations inside the broiler house, and a prolonged exposure to this volatile N compound, may negatively impact health and performance of birds (Anderson et al., 1964; Beker et al., 2004; Miles et al., 2004) as well as the health of farm workers (Pedersen and Selig, 1989; Webb et al., 2005). Environmental issues related to NH_3 emissions are also of increasing concern. The deposition of NH_3 in the environment may impact soil acidity, plant productivity and diversity as well as aquatic life. In addition, NH_3 is a precursor for particulate matter which contributes to diminished air quality (Ritz et al., 2004; Santonja et al., 2017).

The treatment of exhaust air by air scrubbers may reduce NH_3 emitted from the broiler house, but do not prevent the formation of NH_3. The NH_3 release rate from the litter surface is influenced mainly by the near-floor air velocity, temperature, moisture, pH, and total ammoniacal nitrogen (TAN) content of the litter (Liu et al., 2009; Liang et al., 2014). Therefore, source-oriented strategies such as nutrition, management, and ventilation adaptations, by which formation and subsequent emissions of NH_3 can be reduced, should be chosen over end-of-pipe techniques, since the latter do not improve indoor air quality. The N content of broiler excreta and litter is influenced by the composition of the feed as well as the efficiency with which the bird converts dietary N into animal protein. Least-cost feed formulation results in commercial broiler diets with a protein content slightly above the birds' actual requirements (Ferguson et al., 1998). In addition, due to genetic selection, current broiler strains have different protein and AA requirements than a decade ago. The N content of the litter can be reduced by decreasing the N excreted by the birds. This reduction can be achieved by a reduced dietary CP when AA requirements are met (Veldkamp et al., 2012; Santonja et al., 2017). According to the literature, this source based nutritional approach reduced N excretion in broilers, thereby decreasing litter N content and improving litter quality (Aletor et al., 2000; Bregendahl et al., 2002; Belloir et al., 2017; Shao et al., 2018; Lemme et al., 2019; Van Harn et al., 2019). Belloir et al. (2017) found that for each percentage point decrease in dietary CP (and with supplementation of AA), N excretion was reduced by 13%. Previous studies in broilers (Elwinger and Svensson, 1996; Ferguson et al., 1998; Ospina-Rojas et al., 2012; Hernandez et al., 2013) and broiler breeders (Van Emous et al., 2019) have found a reduction in NH_3 emissions when dietary CP was reduced. Within the broiler industry, both mash and pelleted feeds are commonly used. Feed form (FF) influences the development of the gastrointestinal tract as well as feed and water intake (Engberg et al., 2002) and should therefore have an impact on the N and moisture levels of the excreta. Broilers fed pelleted diets resulted in poorer quality litter with higher moisture contents compared to broilers fed mash diets (Huang et al., 2011).

Although the effect of FF and dietary CP content on broiler performance, N utilization, litter quality and NH_3 emissions has been studied to a great extent, the relationships among nutritional strategies, litter quality, and NH_3 concentrations at litter level have insufficiently been quantified. Since broilers are raised for their meat, potential strategies for reducing NH_3 emissions can only be implemented if broiler performance and meat quality are not compromised. The aim of this study was to evaluate the extent to which FF and a reduction in dietary CP content influence broiler performance and welfare, meat yield and quality, N utilization and the subsequent effect on litter quality and NH_3 concentrations at litter level.

2. Materials and methods

2.1. Animal ethics

The study (trial 2018/331) was approved by the Animal Ethics Committee of ILVO (Merelbeke, Belgium) and performed according to the principles for the care of animals used for scientific purposes (Belgian Royal Decree KB29.05.13, 2013).

2.2. Birds, housing and management

A total of 2,232 one-day-old Ross 308 male broilers from a commercial hatchery (Belgabroed, Merksplas, Belgium) were used and randomly allocated to 36 pens (62 birds/pen). Each pen measured 4.41 m^2 and was equipped with 2 feeders and 2 bell drinkers. The birds had free access to feed and water during the experimental period. Each pen contained an equal amount of wood shavings (14 kg/pen) as bedding material and no additional filling or cleaning of the floor was carried out during the trial. All birds were vaccinated against Newcastle Disease. The temperature and humidity schedule applied was according to the Ross 308 standard (Aviagen, 2018). During the first 7 d of age infrared lamps were provided. The lighting schedule applied was 23 h of light during the first 7 d of age, after which 18 h of light were provided until the end of the experimental period. A 3-phase feeding was applied i.e., starter (d 0 to 10), grower (d 10 to 28) and finisher (d 28 to 39). After d 39, the birds remained in their respective pens and received the corresponding finisher diets until slaughter at 42 d of age.

2.3. Experimental diets

The diets (Table 1) were wheat, corn and soybean meal-based and all diets were produced from the same batches of ingredients. The diets within each phase were formulated to be isocaloric. All birds received a starter diet with a CP content of 220.0 g/kg fed in mash or pellet form. During the grower and finisher phases, the diets were formulated to high (H), intermediate (M), and low (L) CP contents. Grower and finisher diets were formulated to CP contents of 205.0 g/kg (H), 187.5 g/kg (M) and 175.0 g/kg (L) and 195.0 g/kg (H), 180.0 g/kg (M) and 165.6 g/kg (L), respectively. Each CP level was fed as mash and pellet form. The aim was to achieve a maximum CP reduction while meeting or exceeding the recommendations for apparent fecal digestible AA according to CVB (2016). Therefore, diets were supplemented with individual synthetic AA to meet the following digestible (dig.) AA ratios: dig. (Met + Cys)-to-dig. Lys ratio = 75%, dig. Thr-to-dig. Lys ratio = 69% (starter) and 66% (grower and finisher), dig. Val-to-dig. Lys ratio = 78%, dig. Arg-to-dig. Lys ratio = 105%, dig. Ile-to-dig. Lys ratio = 66%, dig. Trp-to-dig. Lys ratio = 17%, and dig. Leu-to-dig. Lys ratio = 103%. All diets contained a phytase and non-starch polysaccharide degrading enzyme as well as a coccidiostat. To support intestinal tract development, 10% of the formulated wheat was included as whole wheat for the pelleted diets during the starter and grower phases. All diets were analyzed for moisture content, CP, crude fiber, crude fat, crude ash, and AA content.

2.4. Litter quality and composition

At 14, 28 and 38 d of age, the litter of each pen was visually evaluated according to a 5-point score system (score 0 = completely dry and flaky litter, score 1 = dry, but not easy to move with foot, score 2 = leaves imprint of foot and will form a ball if compacted, but ball does not stay well together, score 3 = sticks to boots and sticks readily in a ball if compacted, score 4 = sticks to boots once the cap or compacted crust is broken) (Welfare Quality Assessment protocol for poultry, 2009). In this study, litter is defined as a mixture of the bedding material (wood shavings), bird excreta, and feathers. In each pen, 5 sampling points were identified: 2 points at the front (in the proximity of the feeders and drinkers), 2 points at the back (away from the feeders and drinkers) and 1 point in the center. On days of litter sampling, approximately 50 g of litter was collected at each sampling point to form a pooled sample of approximately 250 g per pen. Pooled litter samples were freeze-dried, ground to a size of 3 mm and stored until analysis for pH, CP, TAN, and moisture content. To determine the pH of the litter, 10 g of each ground freeze-dried litter sample was mixed with 100 mL of distilled water. After a waiting period of 30 min, the pH was measured by inserting the probe of a Consort multi-parameter analyzer (C3010, Consort bvba, Turnhout, Belgium) into the mixture. Litter temperature was measured at the same 5 points in each pen by inserting the probe of a digital thermometer (testo 110, Alton Hampshire, UK) directly underneath the surface of the litter layer and an average litter temperature per pen was calculated.

2.5. Ammonia concentrations

An indicative litter-level NH_3 concentration measurement system was designed to measure NH_3 concentrations from the litter. The NH_3 concentrations were measured by a Quantum Cascade Laser analyzer from Emerson (CT5200, Cascade Technologies, Stirling, Scotland), coupled to an 8-channel multi-sampler. A total of 8 lids were connected to the analyzer with Teflon tubing, each with a length 50 m. Each lid had a bottom diameter of 52.5 cm, a height of 3 cm at the rim and a height of 14 cm in the center. To measure NH_3 concentrations, a lid was placed directly on the litter surface of a pen and air was drawn through the tubing to the analyzer at an air flow of 80 L/h. The NH_3 concentrations were measured at 3 positions in each pen, i.e., the center as well as the left and right sides at the back of each pen. A 10-min measuring time was allowed at each of the 3 points. Of the 8 lids, 6 lids were used to measure NH_3 concentrations in the pens. To measure background NH_3 concentrations, the 2 remaining lids were placed on clean, fresh wood shavings inside of the broiler house and at the entrance to the broiler house, respectively. The background NH_3 concentrations were measured to ensure that the NH_3 concentrations measured with the lids inside the pens were directly from the litter, and that no environmental NH_3 or possible noise were being measured. As these background measurements were negligible, they were not included in any further calculations. Measurements took place around the age of 5 and 6 weeks. For practical reasons, NH_3 concentrations were measured from half of the pens per treatment on 31 d of age and the other half on 32 d of age (week 5). Measurements were repeated in the same way on 37 and 38 d of age (week 6). From each of the 10-min measurements at 3 points per pen, a mean NH_3 gas concentration (ppm) was calculated from the last 2 min of the steady-state of NH_3 gas concentration. Finally, a mean NH_3 concentration (ppm) was calculated per pen and per treatment. The NH_3 concentration in ppm was converted to a concentration in milligram per cubic meter (mg/m^3) according to the following formula, assuming a temperature of 25 °C and pressure of 1 atm:

$$\text{NH}_3 \text{ concentration } \left(\text{mg}/\text{m}^3\right) = 0.0409 \times \text{NH}_3 \text{ concentration (ppm)} \times \text{molecular weight of NH}_3 .$$

2.6. Bird welfare

Foot and hock lesions were evaluated from 16 birds per pen on 14, 28, and 38 d of age according to a 5-point score system (score 0 = no lesions, score 1 and 2 = minimal evidence of lesions, score 3 and 4 = evidence of lesions) (Welfare Quality Assessment protocol for poultry, 2009).

2.7. Nitrogen digestibility and excreta composition

At 31 d of age, 2 birds per pen from the performance trial were transferred to digestibility units. Each unit consisted of a wire bottom with a plastic tray underneath for the collection of excreta, a feed trough and a drinker. The birds were housed in 36 digestibility units (6 replications per treatment). The digestibility study was performed according to the EU reference method (Bourdillon et al., 1990) and consisted of an adaptation period (from 31 to 34 d of age) and a balance period (from 35 to 39 d of age). During the balance period, feed intake, water intake, and total excreta were determined. The total feed intake was recorded at the end of the balance period, and total excreta were collected after 2 and 4 d of the balance period. Water intake was measured daily by determining the volumetric difference between water supplied and water refused per unit and no corrections were made for evaporation of water. Per digestibility unit, collected excreta was mixed well into one sample and a subsample of each unit was freeze-dried, ground to a size of 1 mm and stored until further analysis for CP, TAN, uric acid, and moisture content. Apparent digestibility coefficients of dietary CP were calculated as described by Macdonald and Bose (1944) with the modification of multiplying CP content of the excreta with the weight ratio of excreta to feed. In addition, the apparent dietary N digestibility coefficients was calculated correcting for uric acid N by the following equation:

$$\text{N digestibility (\%)} = \frac{\frac{\text{CP}_{\text{diet}}}{6.25} - \left[\left(\frac{\text{CP}_{\text{excreta}}}{6.25} - \frac{\text{Uric acid}_{\text{excreta}}}{3}\right) \times \frac{\text{Weight excreta}}{\text{Weight feed}}\right]}{\frac{\text{CP}_{\text{diet}}}{6.25}} \times 100.$$

The N retention (g) was calculated by the following equation:

$$\text{N retention (g)} = \left[\frac{\text{CP}_{\text{diet}}}{6.25} - \left(\frac{\text{CP}_{\text{excreta}}}{6.25} \times \frac{\text{Excreta}}{\text{Feed}}\right) \times 10.\right]$$

At 39 d of age, after the balance period, the birds received the finisher treatments containing 0.4% titanium dioxide as an indigestible marker. After 3 d (at 42 d of age), the birds were euthanized by cervical dislocation, and ileal digesta samples (from 1 cm after Meckel's diverticulum to a point 1 cm before the ileocecal junction) were collected by gently squeezing the contents of the ileum. Digesta collected from the 2 birds of each unit were pooled, freeze-dried, ground to a size of 1 mm and stored until analysis for CP and titanium dioxide. The apparent ileal digestibility (AID) of CP was calculated as described by Ten Doeschate et al. (1993).

2.8. Performance

At 0, 10, 28, and 39 d of age, the birds were weighed per pen and feed consumption per pen was recorded to calculate ADFI, BW, ADG and feed conversion ratio (FCR) (after correcting for mortalities) for each phase.

2.9. Carcass and meat yield and quality

For the determination of the carcass and meat cut yields as well as the different meat quality parameters, 8 birds per treatment were randomly selected at 42 d of age, 3 d after the last weighing moment. The selected birds were individually marked, weighed, and fasted overnight. The fasted birds were transported (according to legal standards) to a commercial slaughterhouse where they were slaughtered and processed. The whole carcasses were transported back to the research facilities of ILVO and stored at 4 °C until the following day when they were weighed, and carcass yield was calculated as eviscerated carcass weight relative to live weight before slaughtering. The carcasses were cut, and the breast, thigh, drumstick and wing yields were calculated as their weight relative to eviscerated carcass weight. Of each carcass, the left breast muscle was selected for measuring temperature, ultimate pH (pHu), color and drip loss. The temperature and pHu were measured using a portable pH meter (Type HI98163 pH meter, Hannah Instruments, electrode FC2323, Woonsocket, Rhode Island, USA). Color was evaluated for the L* (lightness), a* (redness) and b* (yellowness) with a spectrophotometer (Hunterlab MiniScan, Reston, Virginia, USA). A total of 3 pHu and color measurements were taken from each left breast muscle and then averaged. Each left breast muscle was sampled in 2 positions to measure the drip loss after 24 h at 4 °C by applying the EZ-DripLoss method described by Rasmussen and Andersson (1996). The right breast muscle of each carcass was weighed, vacuum packed and stored at −18 °C pending for further analysis of thawing loss, cooking loss and shear force. These breast samples were then thawed at room temperature overnight and weighed to calculate thawing loss as a percentage of the fresh weight. Thawed breast muscles were cooked in a water bath for 45 min at 80 °C, cooled to room temperature, and weighed to calculate cooking loss as a percentage of the thawed weight. The cooked breast muscles were then stored at 4 °C overnight until analysis for shear force. The meat shear force was determined using a Texture Analyzer (TA500, Lloyd Instruments, West Sussex, UK) fitted with a triangular Warner-Bratzler shear. From each cooked breast muscle 10 sub-samples, each with a diameter of 1.27 cm, were cut parallel with the muscle fibers using a circular knife. The maximum force in newton to cut through each of the 10 sub-samples was measured. After eliminating the highest and lowest shear force values, the mean shear force for each breast muscle was calculated from the remaining 8 values.

2.10. Chemical analyses

The CP and moisture contents in the samples of the diets, litter, excreta and ileal digesta were analyzed according to ISO 5983-2 (2009) and Directive 71/393/EEC (1971), respectively. Grower and finisher diets were analyzed for total AA content according to ISO 13903 (2005a), and for total Trp, the MOD.0094 method (2005b) was followed (Metex Nøøvistago, previously Ajinomoto Eurolysine S.A.S.). The crude fat and crude fiber contents of the diets were analyzed according to ISO 6492 (1999) and AOCS Approved Procedure Ba 6a-05 (2005), respectively. Crude ash content of the diets was analyzed according to ISO 5984 (2002). The litter and excreta TAN content was analyzed according to BAM/deel 3/05 (2015). The uric acid content of the excreta was determined using the method of Marquardt (1983). The titanium dioxide content of the ileal digesta and finisher diets was analyzed according to the procedure described by Myers et al. (2004). Feed particle size was determined by wet sieving as described by Millet et al. (2012).

2.11. Statistical analysis

A randomized block design was used (with in total 6 blocks of 6 pens) with a 2 × 3 factorial arrangement: 2 FF, mash and pellet, and 3 CP contents, high (H), intermediate (M) and low (L), resulting in 6 treatments, each with 6 replicates.

For the comparison of the different treatments, data were analyzed with linear mixed models (Bates et al., 2015) using R 3.6.0 for Windows (R Core Team, 2019). The model included FF and dietary CP content as main effects and the interaction between these main effects. If the *P*-value of the interaction was >0.1, the interaction was excluded from the final model. Block (location in the house) was included as a random effect. The experimental unit for all parameters was pen, except for carcass and meat yield and quality parameters, as well as foot and hock lesions scores, for which the experimental unit was bird. For the digestibility parameters, the digestibility unit was the experimental unit. Results were expressed as least square means (lsmeans) and standard error of those means. In case of significant effects, a post hoc pairwise comparison with a Tukey correction was used to determine the significant differences between the treatment means. Significant differences between lsmeans of treatments were declared at a significance level of 5%. Normality of all data were evaluated through visual inspection of quantile–quantile (QQ) plots and histograms of the residuals. For mortality a similar logistic regression model including the same independent variables (Venables and Ripley, 2002) was used. For litter, foot and hock lesion scores, similar ordered logistic regression models including the same independent variables were used, but without a random block effect (Venables and Ripley, 2002).

3. Results

3.1. Experimental diets

Calculated and analyzed values for CP content were generally in good accordance, except for the L CP mash diet of the grower phase which had a CP content of 197.5 g/kg instead of the calculated 175.0 g/kg (Tables 1 and 2). This deviating level of CP content was most likely caused by mixing error or the inclusion of an incorrect protein source.

Table 1
Ingredients and nutrient content of the dietary treatments differing in CP content and feed form[1] (as-fed basis).

Item	Starter	Grower			Finisher		
		H	M	L	H	M	L
Ingredients, %							
Wheat	43.27	49.22	56.04	63.69	54.60	60.76	60.84
Soybean meal	27.25	18.35	14.02	12.40	15.89	13.75	7.00
Corn	15.00	12.00	12.00	10.00	10.00	10.00	10.00
Soybeans	7.00	12.00	8.00	3.29	12.00	7.37	8.50
Sunflower meal	–	0.50	1.00	1.00	–	–	–
Rapeseed meal	–	0.50	1.00	1.00	–	–	–
Wheat middlings	–	–	–	–	0.20	0.20	5.00
Animal fat	2.75	2.35	2.20	2.20	2.50	2.50	2.50
Soybean oil	1.00	1.50	1.44	1.40	1.50	1.50	1.50
Vitamin and mineral premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.78	0.76	0.73	0.69	0.71	0.67	0.75
Dicalcium phosphate	0.72	0.58	0.68	0.78	0.50	0.62	0.54
Na-bicarbonate	0.23	0.32	0.40	0.55	0.27	0.34	0.51
NaCl	0.21	0.17	0.10	0.05	0.18	0.13	0.07
DL-Met	0.29	0.25	0.30	0.33	0.21	0.25	0.30
L-Lysine HCl	0.24	0.26	0.46	0.60	0.24	0.40	0.56
L-Thr	0.15	0.12	0.21	0.26	0.11	0.17	0.24
L-Val	0.05	0.05	0.15	0.22	0.03	0.11	0.20
L-Arg	–	–	0.15	0.28	–	0.11	0.25
L-Ile	–	–	0.09	0.17	–	0.06	0.16
Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase enzyme[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02
NSP enzyme[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrients, g/kg							
CP	220.0	205.0	187.5	175.0	195.0	180.0	165.6
ME, MCal/kg	2.80	2.89	2.89	2.89	2.94	2.94	2.94
Crude fat	74.77	83.47	72.38	62.14	83.95	74.32	76.47
Crude fibre	34.60	34.28	32.53	30.21	32.30	29.89	29.95
Crude ash	44.48	42.80	41.08	39.87	40.09	38.28	38.32
Total Ca	8.50	8.00	8.00	8.00	7.50	7.50	7.50
Total P	5.00	4.71	4.71	4.71	4.41	4.41	4.41
Total Na	1.50	1.60	1.60	1.80	1.50	1.50	1.80
Total Cl	2.20	2.00	2.00	1.93	2.00	2.00	2.00
Calculated dig. AA, g/kg							
dig. Lys	11.50	10.60	10.60	10.60	9.80	9.80	9.80
dig. Thr	7.94	7.10	7.10	7.00	6.57	6.47	6.47
dig. Met	5.64	5.11	5.33	5.48	4.60	4.78	4.99
dig. Met + Cys	8.63	7.95	7.95	7.95	7.35	7.35	7.35
dig. Trp	2.32	2.13	1.86	1.66	2.03	1.80	1.57
dig. Val	8.97	8.27	8.27	8.27	7.64	7.64	7.64
dig. Ile	7.87	7.16	7.00	7.00	6.75	6.47	6.47
dig. Leu	14.22	12.97	11.31	10.05	12.23	10.87	9.40
dig. Arg	12.58	11.42	11.13	11.13	10.67	10.29	10.29
dig. Phe	9.43	8.64	7.51	6.71	8.19	7.28	6.26
dig. His	4.45	3.87	3.43	4.01	4.20	3.72	3.23

H, M, L = high, intermediate and low CP levels, respectively; CP = crude protein; ME = metabolizable energy; NSP = non-starch polysaccharide; AA = amino acid; dig. = digestible.
[1] Feed form included mash and pellet.
[2] Provided the following per kilogram of complete feed: vitamin A, 10,000 IU (retinyl acetate); cholecalciferol, 75 μg; vitamin E, 50 IU (all-rac-alfa-tocopheryl acetate); vitamin K_3, 2.5 mg; vitamin B_1/thiamine mononitrate, 2 mg; vitamin B_2/riboflavin, 5 mg; calcium D-pantothenate, 15 mg; vitamin B_6/pyridoxine hydrochloride, 4 mg; vitamin B_{12}/cyanocobalamin, 0.025 mg; niacinamide, 30 mg; folic acid, 1 mg; biotin/D-(+)-biotin, 0.15 mg; choline chloride, 689.7 mg; iron as iron (II) sulphate (monohydrate), 49.2 mg; copper as copper (II) sulphate (pentahydrate), 20 mg; zinc as zinc oxide, 60 mg; manganese as manganese (II) oxide, 95.9 mg; iodine as calcium iodate (anhydrous), 1.2 mg; selenium as sodium selenite, 0.36 mg; sepiolite, 7 mg; propyl gallate, 2 mg; butylated hydroxytoluene, 3 mg; citric acid, 0.69 mg.
[3] RONOZYME WP (CT), which supplied 2,000 FYT per kilogram of complete feed.
[4] RONOZYME WX 2000 (CT) (Endo-1,4-β-xylanase), which supplied 200 FXU per kilogram of complete feed.

As expected, the mash diets of the starter, grower and finisher phases consisted of coarser feed particles than the pelleted diets (Table 3).

3.2. Litter quality, composition, and ammonia concentrations

A significant treatment effect was observed on litter quality scores at 14 ($P = 0.001$), 28 ($P < 0.001$) and 38 ($P < 0.001$) d of age. Litter quality decreased with time and after the finisher phase, the majority of the pens had a litter quality score of 4. At 14, 28, and 38 d of age, mash treatments generally received better scores than pellet treatments (Fig. 1A, B, and C). At 38 d of age, a decrease in dietary CP content resulted in improved litter quality among the mash treatments: the majority (83%) of the pens from the M and L CP mash treatments received a litter quality score of 3, whereas the same number of pens from the H CP treatment received a litter quality score of 4.

At 28 and 38 d of age, litter from mash treatments had a higher pH than litter from pellet treatments (both $P < 0.001$, Table 4). Only at 38 d of age, dietary treatments affected litter temperature. A significant interaction effect CP × FF ($P = 0.048$), indicated that litter from the pellet treatments and the L CP mash treatment had a higher temperature than litter from the H CP mash treatment and none of these treatments differed significantly from the M CP mash

Table 2
Analyzed values for nutrient content and total AA content of the dietary treatments differing in CP content and feed form.

Item	Starter		Grower						Finisher					
	Mash	Pellet	Mash			Pellet			Mash			Pellet		
			H	M	L	H	M	L	H	M	L	H	M	L
Analyzed nutrients, g/kg														
CP	214.7	222.5	208.9	192.0	197.5	207.4	193.2	176.9	196.2	176.3	165.7	191.2	179.4	166.7
Crude fat	6.43	7.21	85.4	77.8	79.0	82.1	74.9	66.0	88.1	75.4	84.0	86.5	74.5	79.4
Crude fiber	3.99	3.83	45.9	33.0	34.0	30.9	32.3	31.5	31.1	36.1	36.5	38.2	32.8	30.8
Crude ash	5.11	5.10	48.7	47.6	46.8	47.1	47.2	42.5	51.4	47.3	48.5	47.7	45.9	46.6
Analyzed total AA, g/kg														
Lys			12.1	12.1	13.3	12.5	12.6	12.0	10.9	11.2	10.9	11.5	11.5	10.9
Thr			8.0	7.9	8.4	8.2	8.3	7.8	7.2	7.2	7.0	7.7	7.5	7.1
Met			4.9	5.3	5.6	5.1	5.6	5.4	4.5	4.8	4.8	4.6	4.8	4.8
Met + Cys			8.1	8.2	8.6	8.3	8.6	8.2	7.6	7.6	7.5	7.7	7.7	7.5
Trp			2.5	2.2	2.3	2.5	2.3	2.0	2.4	2.2	1.9	2.4	2.2	2.0
Val			9.3	9.3	10.1	9.6	9.8	9.3	8.7	8.6	8.5	8.9	8.8	8.5
Ile			8.1	8.0	8.8	8.4	8.4	7.9	7.7	7.4	7.3	7.9	7.6	7.3
Leu			14.4	12.9	12.9	14.9	13.4	11.6	13.5	12.3	10.7	14.1	12.7	11.0
Arg			12.1	11.9	13.1	12.6	12.4	11.7	11.4	11.0	10.7	11.9	11.3	10.8
Phe			9.5	8.4	8.5	9.8	8.8	7.6	8.9	8.1	7.0	9.3	8.4	7.2
Tyr			6.4	5.6	5.7	6.7	5.9	5.0	6.0	5.4	4.6	6.3	5.6	4.8
His			4.8	4.3	4.3	4.9	4.4	3.8	4.5	4.1	3.6	4.7	4.2	3.6
Ser			9.6	8.6	8.6	9.8	8.8	7.7	8.9	8.3	7.1	9.3	8.4	7.3
Ala			8.2	7.3	7.3	8.4	7.6	6.5	7.6	6.9	6.1	7.9	7.1	6.2
Asp			17.4	14.8	15.0	18.0	15.5	12.4	15.8	14.0	11.4	16.8	14.3	11.6
Glu			41.1	38.3	38.9	42.1	39.1	36.7	40.1	37.8	34.2	40.6	38.4	35.2
Gly			8.1	7.3	7.4	8.3	7.6	6.6	7.6	6.9	6.1	7.9	7.1	6.2
Pro			13.1	12.3	12.4	13.6	12.8	12.2	12.8	12.2	11.2	13.1	12.6	11.8

H, M, L = high, intermediate and low CP levels, respectively; CP = crude protein; AA = amino acid.

Table 3
Average particle size determined by wet sieving for starter, grower and finisher diets.

Item	Starter		Grower						Finisher					
	Mash	Pellet	Mash			Pellet			Mash			Pellet		
			H	M	L	H	M	L	H	M	L	H	M	L
Average particle size, mm	1.24	0.83	1.66	1.67	1.33	0.71	0.68	0.72	1.49	1.56	1.52	0.48	0.51	0.48

H, M, L = high, intermediate and low CP levels, respectively.

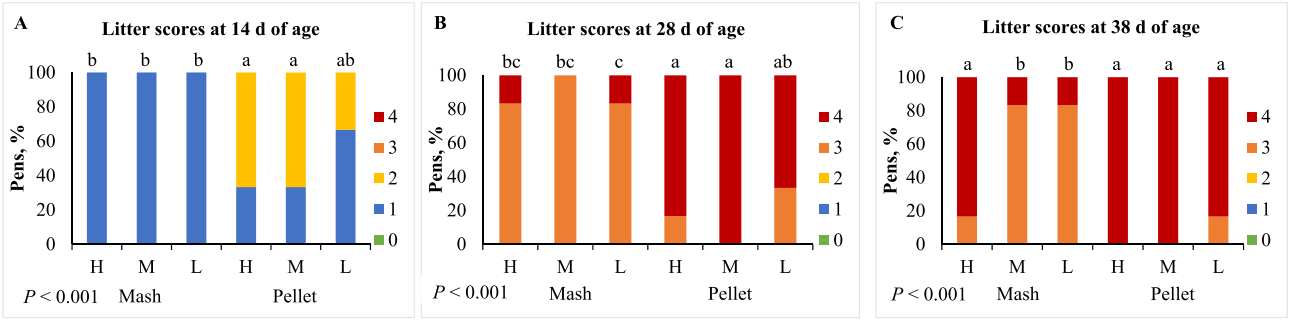

Fig. 1. Percentage of pens with different litter scores for the different treatments: (A) d 14, (B) d 28, (C) d 38 of age. H, M, L stand for high, intermediate and low CP levels, respectively (during the starter phase, dietary CP was the same for all treatments). [a to c] Treatments with different superscripts differ significantly for treatment ($P < 0.05$). Scoring: 0, completely dry and flaky; 1, dry, but not easy to move with foot; 2, leaves imprint of foot and will form a ball if compacted, but ball does not stay together well; 3, sticks to boots and sticks readily in ball if compacted; 4, sticks to boots once the compacted crust is broken (Welfare Quality Assessment protocol for poultry, 2009).

treatment. After the grower and finisher phases, dietary treatments had no effect on litter moisture.

Litter CP content was affected by the interaction CP × FF at 28 and 38 d of age ($P = 0.002$ and $P = 0.007$, respectively). At 28 d of age, litter from the H CP pellet treatment had the highest CP content. At 38 d of age, litter CP content was lowest for the L CP mash and pellet treatments. Among mash treatments, litter CP content was 7.7% and 13.5% lower for M and L CP treatments, respectively, compared to the H CP treatment. Among pellet treatments there was a 7.7% and 19.8% reduction in litter CP content for M and L CP treatments compared the H CP treatment.

At 38 d of age, litter TAN content was affected by FF ($P < 0.001$). If a post-hoc test was performed on FF only, litter TAN content was significantly higher for pellet treatments (average of 2.21 mg/g) compared to mash treatments (average of 1.66 mg/g). At 28 d of age, a significant CP effect was observed

Table 4
Litter analyses at 28 and 38 d of age from litter of treatments differing in CP content and FF.

Item	Mash			Pellets			SEM	*P*-value		
	H	M	L	H	M	L		CP	FF	CP × FF
28 d of age										
pH	6.86^{ab}	7.16^{a}	7.14^{a}	6.71^{b}	6.68^{b}	6.81^{ab}	0.10	0.113	<0.001	–
Temperature, °C	24.7	24.9	24.6	23.7	24.6	24.1	0.5	0.405	0.117	–
CP, %[1]	22.6^{b}	20.9^{c}	21.7^{bc}	24.2^{a}	21.9^{bc}	20.9^{c}	0.4	0.002	<0.001	0.002
TAN, mg/g[1]	1.82	1.37	1.41	1.83	1.65	1.47	0.12	0.003	0.242	–
Moisture, %	48.2	42.1	45.4	48.8	47.3	47.2	2.0	0.164	0.118	–
38 d of age										
pH	6.61^{ab}	6.52^{abc}	6.77^{a}	6.30^{abc}	6.10^{c}	6.15^{bc}	0.12	0.332	<0.001	–
Temperature, °C	23.6^{b}	24.2^{ab}	25.5^{a}	24.0^{a}	26.0^{a}	24.0^{a}	0.6	0.021	0.566	0.048
CP, %[1]	25.9^{ab}	23.9^{c}	22.4^{d}	27.3^{a}	25.2^{bc}	21.9^{d}	0.3	<0.001	0.003	0.007
TAN, mg/g[1]	1.86^{ab}	1.48^{b}	1.63^{ab}	2.36^{a}	2.38^{a}	1.89^{ab}	0.20	0.218	<0.001	–
Moisture, %	46.6	43.9	45.0	46.2	42.8	44.4	1.7	0.096	0.568	–

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; TAN = total ammoniacal nitrogen.
[a to d] Within a row, means with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on pairwise comparisons. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.
[1] Concentrations are reported as a percentage of the dry matter.

Table 5
Average ammonia concentrations measured during 5 and 6 weeks of age from litter of treatments differing in CP content and FF (mg/m^3).

Item	NH_3 during week 5 of age	NH_3 during week 6 of age
Mash		
H	29^{a}	33^{a}
M	16^{b}	23^{b}
L	20^{b}	23^{b}
Pellet		
H	25^{a}	36^{a}
M	11^{b}	19^{b}
L	15^{b}	18^{b}
SEM	3	2
P-value		
CP	<0.001	<0.001
FF	0.055	0.205
CP × FF	–	0.059

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; NH_3 = ammonia.
[a, b] Within a column, means with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on a post-hoc analysis of the main effects only. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.

Table 6
Feed and water intake and water-to-feed ratio (W:F) results from broilers fed finisher diets differing in CP content and FF during the digestibility study from 35 to 39 d of age.

Item	Feed intake, g	Water intake, g	W:F
Mash			
H	1,233^{b}	2,721^{b}	2.20^{a}
M	1,303^{b}	2,481^{b}	1.90^{b}
L	1,291^{b}	2,439^{b}	1.90^{b}
Pellet			
H	1,404^{a}	3,005^{a}	2.16^{a}
M	1,474^{a}	2,764[1,a]	1.86[1,b]
L	1,462^{a}	2,722^{a}	1.86^{b}
SEM	26	135	0.09
P-value			
CP	0.079	0.101	0.003
FF	<0.001	0.018	0.606
CP × FF	–	–	–

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; W:F = water-to-feed intake ratio.
[a, b] Means within a column with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on a post-hoc analysis of the main effects only. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.
[1] Least square means of 5 replicates.

Table 7
Excreta characteristics from broilers fed finisher diets differing in CP content and FF.

Item	Excreta CP, %[1]	Excreta TAN, mg/g[1]	Excreta uric acid, %[1]
Mash			
H	31.7^{a}	1.94^{ab}	8.05^{a}
M	29.8^{a}	1.80^{b}	7.60^{a}
L	26.2^{b}	2.14^{ab}	5.26^{c}
Pellet			
H	32.2^{a}	2.25^{ab}	7.79^{a}
M	29.9^{a}	2.62^{a}	6.40^{b}
L	24.8^{b}	2.43^{ab}	4.76^{c}
SEM	0.6	0.16	0.22
P-value			
CP	<0.001	0.523	<0.001
FF	0.611	0.002	0.001
CP × FF	–	–	–

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; TAN = total ammoniacal nitrogen.
[a to c] Means within a column with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on pairwise comparisons. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.
[1] Concentrations are reported as a percentage of the dry matter.

($P = 0.003$). If a post-hoc test was performed on CP only, litter TAN content was highest for the H CP treatments compared to the reduced CP treatments.

The NH_3 concentrations measured at litter level increased with an average of 31.0% from 5 to 6 weeks of age (Table 5). During weeks 5 and 6, NH_3 concentrations at litter level were affected by dietary CP content (both $P < 0.001$). At 5 weeks of age, among mash and pellet treatments, the NH_3 concentrations was respectively 44.8% and 56.0% lower for the M CP treatments compared to the H CP treatments. At 6 weeks of age, there was a respective 30.3% and 47.2% decrease in NH_3 concentrations for the M CP treatments compared to the H CP treatments. A reduction in dietary CP to the level of the L CP treatments did not result in a further decrease in NH_3 concentrations measured at litter level.

3.3. Nitrogen digestibility and excreta composition

During the present digestibility trial, birds fed pelleted diets had a higher feed intake ($P < 0.001$) and water intake ($P = 0.018$) compared to mash fed birds (Table 6). No significant CP effect was observed on water intake, however, the water-to-feed (W:F) ratio was significantly higher (about 14%) for H CP treatments compared to reduced CP treatments ($P = 0.003$).

Table 8
Protein digestibility and nitrogen retention from broilers fed finisher diets differing in CP content and FF.

Treatments	Apparent CP digestibility, %[1]	N digestibility corrected for uric acid N, %[1]	N retention, g	AID of CP, %
Mash				
H	63.6^{b}	87.5^{ab}	22.2^{c}	81.7^{a}
M	64.7^{b}	88.0^{a}	22.9^{bc}	77.0^{ab}
L	68.3^{a}	86.1^{ab}	25.6^{a}	69.1^{b}
Pellet				
H	60.0^{c}	85.8^{ab}	20.5^{d}	80.3^{a}
M	62.6^{bc}	85.1^{ab}	20.0^{d}	70.4^{b}
L	65.4^{ab}	83.4^{b}	23.8^{b}	69.6^{b}
SEM	0.8	1.1	0.2	2.0
P-value				
CP	<0.001	0.153	<0.001	<0.001
FF	<0.001	<0.001	<0.001	0.146
CP × FF	–	–	0.034	–

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; N = nitrogen; AID = apparent ileal digestibility.
[a to d] Means within a column with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on pairwise comparisons. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.
[1] Determined by total collection of the excreta.

Excreta characteristics from broilers fed finisher diets are presented in Table 7. The L CP treatments had a lower excreta CP content compared to H and M CP treatments ($P < 0.001$). After a post-hoc test was performed on CP only, there was a reduction in excreta CP content for the M CP as well as L CP treatments compared to the H CP treatments. The M CP pellet treatment had a higher excreta TAN content than the M CP mash treatment ($P = 0.002$) and neither differed significantly from the H and L CP treatments. However, after a post-hoc analysis was performed on FF only, pellet treatments had a higher excreta TAN content than mash treatments. The concentration of uric acid in the excreta was affected by both CP ($P < 0.001$) and FF ($P = 0.001$). After a post-hoc analysis was performed on the main effects CP and FF only, there was a clear decrease in excreta uric acid concentration with a reduction in dietary CP. In addition, mash treatments had a higher concentration of uric acid in the excreta compared to pellet treatments.

Table 8 presents the N digestibility and retention results from the digestibility study. Apparent CP digestibility coefficients determined by total collection of the excreta were affected by CP and FF (both $P < 0.001$). After a post-hoc analysis was performed on both CP and FF, apparent CP digestibility was higher for birds fed the mash treatments compared to the pellet-fed birds. Furthermore, these values were highest for the L CP treatments as well as M CP pellet treatment, followed by the H CP treatments and the M CP mash treatment. The N digestibility corrected for uric acid N was affected by FF ($P < 0.001$), and after a post-hoc test was performed on FF only, these digestibility coefficients were higher for birds fed mash diets compared to those fed pelleted diets. The interaction CP × FF ($P = 0.034$) influenced N retention, which was highest for the L CP mash treatment followed by the L CP pellet treatment. The H and M CP pellet treatments had the lowest N retention. Only a CP effect was observed for apparent ileal CP digestibility ($P < 0.001$). After a post-hoc test was performed on CP only, apparent ileal CP digestibility was higher for the H CP fed birds compared to birds fed the reduced CP treatments.

3.4. Bird welfare

At 14 d of age, the occurrence of foot and hock lesions were negligible (data not shown), while a higher prevalence of foot and hock lesions were observed after the grower and finisher phases (Figs. 2 and 3). In general, hock lesions were less severe than foot lesions. At 28 d of age, the prevalence of foot lesions was highest for the H CP pellet treatment, followed by the M CP pellet treatment ($P < 0.001$). There was no significant difference in the prevalence of foot lesions between the mash and L CP pellet treatments (Fig. 2A). At 38 d of age, the prevalence of foot lesions was highest for the H

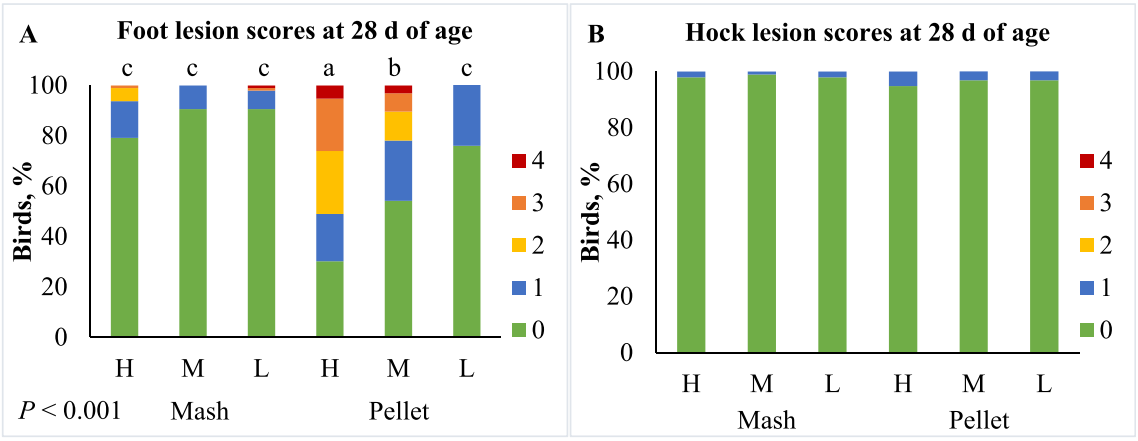

Fig. 2. Percentage of birds with different foot and hock lesion scores at 28 d of age for treatments differing in dietary CP content and feed form: (A) foot lesions, (B) hock lesions. H, M, L stand for high, intermediate and low CP levels, respectively. [a to c] Treatments with different superscripts differ significantly for treatment ($P < 0.05$). Scoring: 0, no evidence of foot or hock lesions; 1 and 2, minimal evidence of foot or hock lesions; 3 and 4, evidence of foot or hock lesions (Welfare Quality Assessment protocol for poultry, 2009).

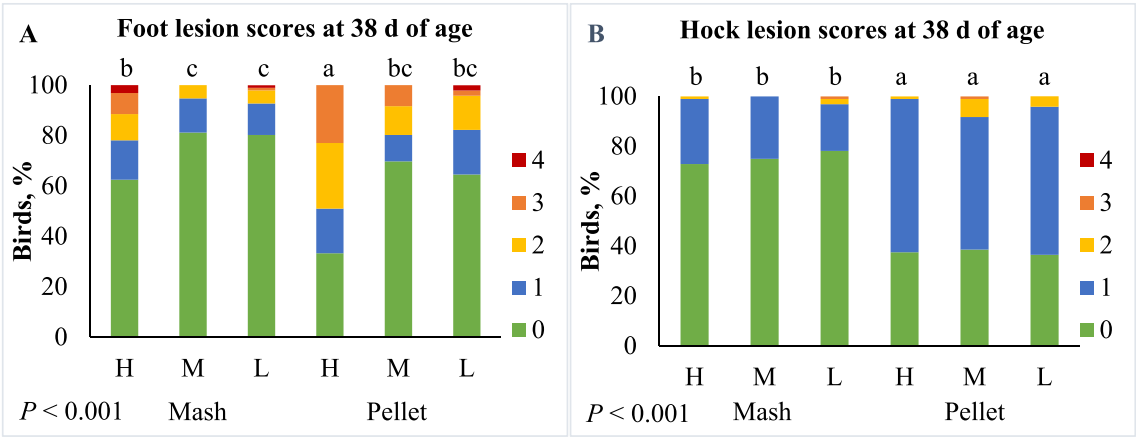

Fig. 3. Percentage of birds with different foot and hock lesion scores at 38 d of age for treatments differing in dietary CP content and feed form: (A) foot lesions, (B) hock lesions. H, M, L stand for high, intermediate and low CP levels, respectively. [a to c] Treatments with different superscripts differ significantly for treatment ($P < 0.05$). Scoring: 0, no evidence of foot or hock lesions; 1 and 2, minimal evidence of foot or hock lesions; 3 and 4, evidence of foot or hock lesions (Welfare Quality Assessment protocol for poultry, 2009).

Table 9
Productive performance of broilers fed diets differing in CP content (during the grower and finisher phases) and FF.[1]

Item	Mash			Pellets			SEM	*P*-value		
	H	M	L	H	M	L		CP	FF	CP × FF
Starter (0 to 10 d of age)										
ADFI, g/bird per day	22.4^{b}	22.3^{b}	22.5^{b}	24.3^{a}	24.4^{a}	24.1^{a}	0.3	–	<0.001	–
BW on d 10, g/bird	215^{b}	214^{b}	216^{b}	258^{a}	259^{a}	256^{a}	3	–	<0.001	–
ADG, g/bird per day	16.1^{b}	15.9^{b}	16.2^{b}	20.4^{a}	20.4^{a}	20.2^{a}	0.3	–	<0.001	–
FCR, g/g	1.39^{b}	1.40^{b}	1.39^{b}	1.20^{a}	1.20^{a}	1.20^{a}	0.01	–	<0.001	–
Grower (10 to 28 d of age)										
ADFI, g/bird per day	84.2^{b}	83.7^{b}	82.8^{b}	91.0^{a}	91.4^{a}	88.7^{a}	0.9	0.051	<0.001	–
BW on d 28, g/bird	1175^{c}	1195^{bc}	1252^{b}	1423^{a}	1403^{a}	1363^{a}	16	<0.001	<0.001	<0.001
ADG, g/bird per day	53.3^{d}	54.5^{cd}	57.5^{c}	64.7^{a}	63.5^{ab}	61.5^{b}	0.8	<0.001	<0.001	<0.001
FCR, g/g	1.58^{c}	1.54^{bc}	1.44^{ab}	1.41^{a}	1.44^{ab}	1.44^{ab}	0.02	<0.001	<0.001	<0.001
Finisher (28 to 39 d of age)										
ADFI, g/bird per day	163.3^{d}	166.1^{cd}	164.2^{cd}	176.9^{ab}	180.1^{a}	171.0^{bc}	1.6	0.454	<0.001	0.041
BW on d 39, g/bird	2,147^{d}	2,222^{cd}	2,261^{c}	2,635^{a}	2,640^{a}	2,515^{b}	23	0.001	<0.001	<0.001
ADG, g/bird per day	88.4^{c}	93.3^{c}	91.7^{c}	110.3^{a}	112.5^{a}	104.7^{b}	1.3	0.016	<0.001	0.001
FCR, g/g	1.85^{b}	1.78^{b}	1.79^{b}	1.60^{a}	1.60^{a}	1.63^{a}	0.02	0.004	<0.001	0.014
Whole period (0 to 39 d of age)										
ADFI, g/bird per day	90.6^{c}	91.2^{c}	90.3^{c}	98.1^{ab}	99.3^{a}	95.3^{b}	0.8	0.015	<0.001	–
ADG, g/bird per day	53.7^{d}	55.6^{cd}	56.6^{c}	66.2^{a}	66.3^{a}	63.1^{b}	0.6	0.001	<0.001	<0.001
FCR, g/g	1.69^{c}	1.64^{bc}	1.60^{b}	1.48^{a}	1.50^{a}	1.51^{a}	0.02	<0.001	<0.001	<0.001

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; ADFI = average daily feed intake; BW = body weight; ADG = average daily gain; FCR = feed conversion ratio.
[a to d] Within a row, means with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on pairwise comparisons. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.
[1] During the starter phase, all birds received diets with an equal amount of CP (220.0 g/kg). Diets differing in CP content (H, M, L) were fed from the grower phase onwards.

CP pellet treatment, followed by the H CP mash treatment. Among the mash treatments there was a higher prevalence of foot lesions for the H CP compared to the M and L CP treatments ($P < 0.001$). The M and L CP pellet treatments did not differ significantly from the mash treatments (Fig. 3A). The prevalence of hock lesions at 38 d of age was highest for pellet treatments compared to mash treatments ($P < 0.001$, Fig. 3B).

3.5. Performance

During the starter phase all birds received feed with the same CP content and, as expected, only feed form had a significant effect on ADFI, BW, ADG and FCR ($P < 0.001$, Table 9). Birds receiving pelleted obtained the best performance.

During the grower phase pellet treatments had a higher ADFI compared to mash treatments (FF, $P < 0.001$). An interaction effect was observed between FF and CP level for BW, ADG and FCR ($P < 0.001$). All birds fed pellet treatments had a higher BW at 28 d of age compared to birds from mash treatments. Birds fed pellet treatments had a higher ADG than birds fed mash treatments and, among the pellet treatments, the L CP treatment had a lower ADG than the H CP treatment and neither differed significantly from the M CP treatment. The FCR from the H CP pellet treatment was significantly lower (better) than FCR from the H and M CP mash treatments and did not differ significantly from the H and L CP pellet treatments.

During the finisher phase, an interaction effect was observed between FF and CP for ADFI ($P = 0.041$), BW ($P < 0.001$), ADG ($P = 0.001$), and FCR ($P = 0.014$). The ADFI for the M CP pellet treatment was significantly higher compared to the mash treatments as well as the L CP pellet treatments and did not differ significantly from the H CP pellet treatment. No significant differences for ADFI were observed among the mash treatments. Final BW was highest for birds from the H and M CP pellet treatments, followed by the L CP pellet treatment. Birds from the H CP mash treatment had a lower BW than the L CP mash

treatment and neither treatment differed significantly from the M CP mash treatment. The ADG was highest for the H and M pellet treatments, followed by the L CP pellet treatment. Mash treatments resulted in lowest ADG during the finisher period. Finally, birds fed pelleted diets had a better FCR compared to birds fed mash diets.

For the overall period, only main effects were observed for ADFI. After a post-hoc test was performed on CP and FF only, ADFI was higher for pellet treatments compared to mash treatments ($P < 0.001$). In addition, L CP treatments resulted in decreased ADFI compared to M CP treatments ($P = 0.015$) and neither treatment differed significantly from the H CP treatments. An interaction effect was observed for ADG and FCR (both $P < 0.001$). Overall ADG was highest for birds from the H and M CP pellet treatments, followed by the L CP pellet treatment. Birds from the H CP mash treatment had a lower ADG than birds from the L CP mash treatment and neither differed significantly from the M CP mash treatment. Pelleted treatments resulted in improved FCR compared to mash treatments. The FCR did not differ among the pellet treatments, but among mash treatments, the L CP treatment resulted in a better FCR than the H CP treatment.

3.6. Carcass and meat yield and quality

Values for BW at slaughter, carcass yield and yields of the different meat cuts are presented in Table 10. An effect of FF was observed for carcass yield. After a post-hoc test was performed on FF only, pellet treatments resulted in a higher carcass yield compared to mash treatments ($P = 0.001$). The dietary treatments had no effect on the different meat cut yields.

The results of the different meat quality parameters are summarized in Table 11. Thawing loss was affected by both FF ($P = 0.007$) and CP ($P = 0.024$). After a post-hoc test was performed on the main effects only, breast meat from mash treatments had a higher thawing loss than those from pellet treatments; however, the post-hoc analysis showed no further distinction between the different CP treatments. Drip loss was affected by the interaction CP × FF ($P = 0.006$), with breast meat from the H CP pellet treatment having a higher drip loss than those from the H CP mash treatment. Neither treatment differed significantly from the M and L CP mash and pellet treatments. The pHu was below 6 for all treatments and was affected by the interaction CP × FF ($P < 0.001$). The H CP mash treatment had the lowest pHu compared to the other treatments. The lightness of the meat was affected by CP ($P < 0.001$) and after a post-hoc test was performed on CP only, meat from treatments with the lowest CP content had the lightest color (highest L* values). Values for b* was affected by the interaction CP × FF ($P < 0.05$). Meat from the M CP pellet treatment had a higher b* value than breast meat from the M CP mash treatment, but the respective treatments did not differ significantly from the other treatments. Shear force was affected by CP content ($P < 0.001$) and after a post-hoc analysis was performed on CP only, breast meat from treatments containing the lowest CP content had the highest shear force.

Table 10
Meat yield parameters of broilers fed diets differing in CP content and FF.

Item	BW at slaughter, g	Carcass yield, %	Breast meat yield, %	Thigh yield, %	Drumstick yield, %	Wings yield, %	Waste, %
Mash							
H	2658[b]	65.7[b]	32.1	27.0	13.7	10.5	15.9
M	2665[b]	66.3[ab]	31.5	27.0	13.7	10.6	16.7
L	2964[ab]	66.6[ab]	33.5	26.5	13.7	10.1	15.6
Pellets							
H	3247[a]	68.8[a]	33.6	26.3	13.1	10.2	16.2
M	3137[a]	68.1[ab]	33.7	26.1	13.3	10.2	16.1
L	3084[a]	67.4[ab]	32.2	27.3	13.6	10.7	15.8
SEM	94	0.7	0.9	0.4	0.4	0.3	0.4
P-value							
CP	0.027	0.925	0.965	0.564	0.825	0.993	0.182
FF	<0.001	0.001	0.263	0.155	0.186	0.812	1.000
CP × FF	0.030	–	–	0.068	–	–	–

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; BW = body weight.
[a, b] Within a column, means with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on pairwise comparisons. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.

Table 11
Meat quality parameters of broilers fed diets differing in CP content and FF.

Item	Thawing loss, %	Cooking loss, %	Drip loss, %	pHu	L*	a*	b*	Shear force, N
Mash								
H	9.0[ab]	16.5	3.1[b]	5.65[b]	56.3[b]	7.7	16.6[ab]	9.1
M	8.9[ab]	17.5	3.4[ab]	5.84[a]	56.9[ab]	7.4	16.1[b]	8.5
L	10.6[a]	19.0	4.6[ab]	5.87[a]	59.6[ab]	7.1	16.8[ab]	11.2
Pellets								
H	7.2[b]	17.6	5.5[a]	5.93[a]	57.5[ab]	7.5	16.8[ab]	9.5
M	7.4[b]	17.3	4.2[ab]	5.92[a]	58.1[ab]	7.8	17.4[a]	8.5
L	9.0[ab]	18.3	3.6[ab]	5.88[a]	60.2[a]	6.7	16.7[ab]	11.0
SEM	0.7	0.9	0.6	0.04	0.9	0.5	0.3	0.7
P-value								
CP	0.024	0.119	0.128	<0.001	<0.001	0.219	0.199	<0.001
FF	0.007	0.924	0.001	<0.001	0.146	0.808	0.505	0.898
CP × FF	–	–	0.006	<0.001	–	–	0.039	–

H, M, L = high, intermediate and low CP levels, respectively; SEM = standard error of the mean; CP = crude protein; FF = feed form; pHu = ultimate pH; L* = lightness; a* = redness; b* = yellowness.
[a, b] Means within a column with different superscripts differ significantly ($P < 0.05$). Reported values are least square means based on pairwise comparisons. If the *P*-value of the interaction was >0.1, the interaction was excluded from the model.

4. Discussion

The increase in NH_3 concentrations measured at litter level from 5 to 6 weeks of age may have been a result of litter CP and TAN build-up between these 2 measuring periods. Although the method used to measure the NH_3 concentrations at litter level is only indicative of NH_3 emission, the measured values still give meaningful insight into possible effects under commercial conditions. Lowering the dietary CP content by 17.5 g/kg during the grower phase and 15.0 g/kg during the finisher phase reduced NH_3 concentrations measured at litter level for week 5 (a 44.8% reduction among the mash treatments and a 56.0% reduction among the pellet treatments) and week 6 (a 30.3% reduction among the mash treatments and a 47.2% reduction among the pellet treatments). However, a further reduction in dietary CP content by about 30.0 g/kg in total during both phases (the 30.0 g/kg reduction did not apply to the L CP mash grower diet) had no additional effect on NH_3 concentrations. At week 5, this non-linear response in NH_3 concentrations corresponded to the TAN content of the litter at 28 d of age. At 38 d of age, there was only a numerical decrease in TAN content of the litter with decreasing dietary CP content. In addition, NH_3 concentrations did not coincide with the gradual decrease in CP and uric acid content of the excreta as well as the CP content of the litter at 38 d of age. This indicates that, once the dietary CP content was decreased by 15.0 g/kg during the finisher phase, the concentration of uric acid was probably not the most determining factor for the formation of NH_3.

Correcting CP digestibility for uric acid production showed that digestibility was not different between treatments differing in dietary CP content, but that the difference in N excretion between the H CP and reduced CP treatments must have been caused by the higher uric acid excretion coming from catabolism of excess absorbed AA. The higher W:F ratio in H CP treatments agrees with Chrystal et al. (2020), and may relate to the higher uric acid excretion. In addition, H CP diets have a higher overall soybean and soybean meal content than the reduced CP treatments. These feedstuffs are rich in potassium, which can lead to a higher water intake (Eichner et al., 2007). This study thus shows a higher importance of excess AA absorption compared to reduced CP digestibility as a trigger for the volatilization of NH_3. The conversion of uric acid to NH_3 is evidently much easier than from indigested or microbial protein to NH_3. A higher W:F ratio may result in more water being excreted by the bird, and consequently, an increased moisture content of the excreta and litter. The differences in W:F ratio was not translated into differences in litter moisture content at 28 and 38 d of age.

The effect of FF works in a different way: even though the CP digestibility was poorer with pelleted diets, the concentrations of NH_3 measured were similar, and even tended to be lower than those of mash diets, despite a higher TAN content in the litter and excreta of pellet treatments. This leads to the assumption that also a physical barrier was influencing the formation and volatilization of NH_3. Due to deteriorating litter quality with time, a crust formed on top of the litter layer near the drinkers and the feeders. This crust leads to anaerobic conditions which are less favorable for the conversion of uric acid to NH_3 and the pH remains low. The lower litter pH and NH_3 concentrations coinciding with more pronounced crust formation with the pelleted diets are confirming the idea of physical rather than chemical barriers for NH_3 volatilization. In addition, at a lower pH, the amount of free ammonia that can be volatilized from the litter decreases (Srinath and Loehr, 1974), which may explain why NH_3 concentrations from pellet treatments tended to be lower, even though these treatments had a higher TAN content in the litter. As the optimal pH for uricase activity is 9.5 (Machida and Nakanishi, 1980), a pH of below 7 would imply that the volatilization of NH_3 from the litter is very low or negligible. The overall low litter pH in the current study may be due to sampling issues, e.g., the pooling of more friable litter samples from the back of the pen with caked litter samples near the drinkers and feeders.

The development of foot and hock lesions in broilers is mainly linked to elevated levels of moisture, N and NH_3 in the litter (De Jong et al., 2014; Dunlop et al., 2016; Shao et al., 2018; Lemme et al., 2019; Van Harn et al., 2019). The downside of the litter crust may therefore be the avoidance of moisture evaporation, leading to wetter litter and the concomitant foot and hock lesions. In addition, elevated levels of litter TAN content for H CP treatments at 28 d of age and for pellet treatments compared to mash treatments at 38 d of age further justify the observed differences in foot and hock lesions.

If NH_3 reducing strategies in broilers are to be implemented in practice, they should not affect performance, meat yield and meat quality negatively. Pelleted diets led to increased feed intake and a higher and more efficient lean growth with a higher carcass yield. At first sight, this may seem a paradox since CP digestibility as well as N retention was lower with pellets, but the faster growth has led to a lower proportion of maintenance requirements, hence compensating for the less efficient CP digestion and AA metabolization. The better performance on pellets versus mash agrees with several studies reporting on the effect of feed form on broiler performance (Zang et al., 2009; Dozier et al., 2010; Chewning et al., 2012; Serrano et al., 2012). Attia et al. (2014) found gizzard weight to decrease and abdominal fat percentage to increase when broilers were fed pelleted diets compared to mash diets. In this study, during the starter and grower phases, 10% of the formulated wheat was supplemented as whole wheat for the pelleted diets. As in practice, this was done to provide more structure to the pelleted diets to support the development of the intestinal tract which, in turn, might have influenced the digestibility of the pelleted diets. Although not measured in this study, a lighter intestinal tract and higher abdominal fat percentage may also have contributed to a higher carcass yield in pellet-fed birds compared to mash fed birds. After the performance trial, the aim is to select birds for slaughter that represent the average BW of the treatment. The slightly different treatment effects observed for BW at slaughter (42 d of age) compared to BW at 39 d of age, may be due to the random selection of the birds as well as the fact that only 8 broilers per treatment were selected, which decreased the statistical power.

When feeding low CP diets, some studies found that broiler performance decreased, even though AA were supplemented to meet requirements (Bregandahl et al., 2002; Pesti, 2009; Namroud et al., 2010; Lemme et al., 2019; Chrystal et al., 2020, 2021). In the present study, the performance of the L CP pellet treatments could not be maintained. The reduced performance could be explained by insufficient levels of non-essential AA in the diets of these treatments. Glycine and serine are likely to become limiting in low CP plant-based diets and a deficiency may impair broiler performance (Siegert and Rodehutscord, 2019). Some studies have indicated that glycine supplementation to low CP diets can have beneficial effects on broiler performance (Dean et al., 2006; Ospina-Rojas et al., 2012; Kriseldi et al., 2017). Recommended levels for glycine and serine in broiler diets vary from 12.5 g/kg for 0–21 d of age (NRC, 1994) to 15.0 g/kg for 0–14 d of age (CVB, 2018). In the present study, the total glycine + serine levels of the L CP treatments were 16 and 14.3 g/kg for the mash and pellet treatments of the grower phase and 13.2 and 13.5 g/kg for the mash and pellet treatments of the finisher phase. The ratios of glycine + serine to lysine in the L CP treatments were 1.2 g/kg and below the ratios recommended by CVB (2018). Reducing the dietary CP content had no effect on the different meat yield parameters and this agrees with Aletor et al. (2000), Belloir et al. (2017), and Van Harn et al. (2019).

Although meat yield is important, meat quality should not be neglected as these parameters may influence consumers when deciding to purchase meat. Based on the CIE76 color difference formula, a value of approximately 2.3 corresponds to a just noticeable difference (JND) and is visually perceptible. In this study, among mash and pellet treatments, color differences between the L CP and H and M mash treatment and L CP and H CP pellet treatment were visually perceptible. Lighter meat color with a decreasing dietary CP content supports the findings of Corzo et al. (2002) and Yalçin et al. (2010). These observations for L* and shear force may be linked to thawing loss or muscle fiber size. Thawing loss was numerically higher for the L CP treatments. In the study of Yalçin et al. (2010), a lower myofiber density (because of larger myofiber) was obtained in broilers fed a high CP diet. In pigs, animals with larger muscle fibers have a higher protein turnover (Nissen et al., 2014) and this may positively influence meat tenderness (Kristensen et al., 2002). In a study by Berri et al. (2007), a larger muscle fiber diameter was associated with a slower post-mortem pH decrease and a darker color.

To conclude, the present study shows that although mash diets are favorable when considering the overall litter quality and welfare of the birds, they cannot maintain the same broiler performance and slaughter yield as pelleted diets. A dietary CP reduction positively influences litter quality and bird welfare and leads to a reduction in NH_3 concentrations measured at litter level; however, a CP reduction to 165.6 g/kg in the finisher phase led to decreasing performance and may not be advisable. Based on the results of this experiment, the optimal level of dietary CP might be between the intermediate level (187.5 g/kg for the grower and 180.0 g/kg for the finisher) and low level (175.0 g/kg for the grower and 165.6 g/kg for the finisher) treatments.

Through a source-oriented approach, the formation and volatilization of NH_3 can be reduced. This benefits not only the environment, but also the farmer and animals by optimizing the indoor climate. Although the effect of the most promising strategies from this study on NH_3 emissions from the broiler house needs to be studied on a larger scale and under practice conditions, the results seem promising and already provide insight into the effect of a dietary CP reduction and feed form on NH_3 emissions.

Author contributions

Madri Brink: investigation, visualization, writing original draft, project administration, conceptualization**,** methodology. **Geert Janssens:** supervision, writing – review & editing. **Peter Demeyer:** writing – review & editing, funding acquisition, project administration. **Özer Bağci:** writing – review & editing, project administration. **Evelyne Delezie:** supervision, writing – review & editing, funding acquisition, project administration, conceptualization, methodology.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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