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Original Research Article

Does feeding more phases reduce ammonia concentrations from broiler litter?

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ABSTRACT

We compared a 3- and 5-phase feeding program in terms of ammonia concentrations measured at litter level, litter composition and quality, nitrogen (N) utilization, performance, welfare, and meat deposition in broilers. A total of 744 Ross 308 male broilers was divided into 2 treatments, each with 6 replicates. The 3-phase diets were formulated to CP and digestible lysine contents of 205 and 11.5 g/kg (0 to 9 d), 195 and 10.8 g/kg (9 to 24 d), and 183.3 and 10.15 g/kg (24 to 39 d), respectively. The 5-phase diets had additional phases from 17 to 24 d and 32 to 39 d with CP and digestible lysine contents of 188 and 10.4 g/kg and 177 and 9.8 g/kg, respectively. Feeding 5 phases reduced the NH_3 concentrations at litter level by 37.95% and 20.81% at 23 ($P < 0.05$) and 37 d of age ($P < 0.05$), respectively. There was a tendency for a lower litter total ammoniacal nitrogen concentration for the 5-phase treatment than the 3-phase treatment at 24 and 39 d of age ($P < 0.1$ for both). Total N concentration of the litter also tended to be lower for the 5-phase treatment at 39 d of age ($P < 0.1$). The lower NH_3 coincided with a lower average litter pH at 24 and 32 d of age ($P < 0.05$ for both). Birds fed the 5-phase treatments had a lower incidence of foot lesions at 24 d of age ($P < 0.001$). Performance as well as carcass yield could be maintained, except for FCR, which was higher for the 5-phase treatment between 17 and 24 d of age and the overall period ($P < 0.05$ and < 0.001, respectively). The impact of the 5-phase treatment regarding the utilization of N (digestibility, excretion, and retention) was less clear, however the NH_3 volatilized from the litter was significantly reduced. Feeding 5 phases may therefore potentially reduce the NH_3 emitted from broiler houses which has several environmental and health benefits. Although the 5-phase treatment resulted in a significant decrease in feed efficiency, growth performance and meat deposition were maintained.

1. Introduction

It is well known that broilers' CP and AA requirements decrease with age in terms of dietary concentrations, however, it is hard to predict the exact daily nutrient requirements of the broilers because it is affected by several environmental factors, disease challenges, strain, sex, and activity. Despite the major advances in the broiler industry in terms of genetics and production efficiency, it seems as though the nutrient recommendations did not keep up. Although the requirements provided by the NRC (1994) and recommendations provided for Ross 308 broilers (Aviagen, 2019) are useful as a reference, most commercial broiler producers in the EU apply a 3- or 4-phase feeding program with dietary CP levels lower than those recommended by the above-mentioned references (Curial et al., 2018). According to Ritz et al., (2004), in poultry it is estimated that approximately two-thirds of the nitrogen (N) consumed by the bird is excreted as uric acid and as undigested feed protein in the feces. In the litter, the uric acid and undigested proteins are degraded in a series of complex processes mediated by microbes, giving rise to ammonia (NH_3) as one of the by-products (Groot Koerkamp, 1994). Ammonia volatilized from the litter may negatively impact bird performance and health (Anderson et al., 1964; Miles et al., 2004). After emitted from the broiler house, NH_3 is a precursor of particulate matter which contributes to air pollution and may travel large distances. Once deposited in the environment, NH_3 may also contribute to eutrophication and the

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acidification of soil and water, which in turn negatively impacts the productivity of crops, plant diversity, and aquatic life (Santonja et al., 2017).

One of the nutritional strategies which is recommended to reduce the excess N excreted by broilers is to increase the number of feeding phases during the rearing period, aiming to meet the nutritional requirements of the birds' more closely during specific periods of rearing (Pope et al., 2004; Santonja et al., 2017). The ideal phase feeding program should reduce the over- or undersupply of AA, in order to increase N utilization efficiency so that technical performance and meat yield are not sacrificed. As the amount of N excreted can be reduced, phase feeding could potentially reduce the amount of NH_3 volatilized from the litter. In addition, the phase feeding program should be practically and logistically feasible.

The objective of this study was to compare a 3- and 5-phase feeding program in terms of NH_3 concentrations at litter level, litter quality, N-utilization, welfare, performance, as well as meat yield and quality of broiler chickens.

2. Materials and methods

2.1. Animal ethics statement

The trial (2020/375) and all experimental procedures applied were approved by the Animal Ethics Committee of ILVO (Merelbeke, Belgium). All procedures involving live birds handling, management, and health care were performed according to the principles for the care of animals used for scientific purposes (Belgian Royal Decree KB.29.05.13, 2013).

2.2. Animals and husbandry

A total of 744 one-day-old Ross 308 male broilers were obtained from a commercial hatchery (Belgabroed, Merksplas, Belgium). Upon arrival, the birds were weighed and randomly placed in 12 separate floor pens (62 birds/pen). Each pen had a surface area of 4.41 m^2 and was equipped with 2 feeders and 2 bell drinkers. Wood shavings (14 kg/pen) were used as bedding material. The feed and water were provided ad libitum. All birds were vaccinated against Newcastle Disease. The house was environmentally controlled, and the temperature and humidity schedule applied was in line with the recommendations given in the Ross 308 management handbook (Aviagen, 2018). Infrared lamps were provided during the first 7 d of age. 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.

2.3. Dietary treatments

The trial consisted of 2 treatments, a 3- and a 5-phase feeding program. The aim was to formulate a standard type 3-phase feeding program and a 5-phase feeding program to meet the nutritional needs of the birds more closely during specific periods of the trial period. In this study, the CP levels of the 'standard' 3-phase feeding program were based on CP levels which are currently generally applied in commercial broiler production in the EU. These CP levels are lower than CP levels recommended by NRC (1994) and the breed recommendations by Aviagen (2019). There were 6 replicate pens per treatment. The ingredients and calculated nutrient concentrations of the respective phases are presented in Table 1. The 3-phase treatment consisted of the Starter, Grower 1 and Finisher 1 phases. The 5-phase treatment had additional Grower 2 and Finisher 2 phases with lower CP and digestible lysine contents than the Grower 1 and Finisher 1 phases. When necessary, the diets were supplemented with individual synthetic AA to meet the following digestible (dig.) AA ratios: (dig.Met + dig.Cys):dig.Lys = 75%; dig.Thr:dig.Lys = 67%; dig.Val:dig.Lys = 78%; dig.Arg:dig.Lys = 103%; dig.Ile:dig.Lys = 65%; dig.Trp:dig.Lys = 17%; dig.Leu:dig.Lys = 103%. The diets were formulated to meet or exceed the recommendations for apparent fecal digestible AA according to CVB (2016) as well as industry averages (based on personal communication with project

Table 1
Ingredients and calculated nutrient content (as-fed-basis) of the 3-phase (Starter, Grower 1, and Finisher 1) treatment and 5-phase (Starter, Grower 1, Grower 2, Finisher 1, and Finisher 2) treatment.

Item	Starter	Grower 1	Grower 2	Finisher 1	Finisher 2
	0 to 9 d	9 to 17 d	17 to 24 d	24 to 32 d	32 to 39 d
Ingredients, %					
Wheat	58.69	60.83	61.54	61.98	62.60
Soybean meal	23.71	22.15	18.96	16.97	14.18
Corn	8.00	7.06	7.44	7.67	8.00
Soybeans	4.00	3.00	4.81	5.94	7.52
Vitamin and mineral premix[1]	1.00	1.00	1.00	1.00	1.00
Animal fat	1.00	2.56	2.74	2.85	3.00
Dicalcium phosphate	0.76	0.59	0.53	0.50	0.45
Soybean oil	0.50	0.50	0.70	0.83	1.00
Limestone	0.47	0.52	0.50	0.48	0.46
L-Lysine HCl	0.40	0.38	0.38	0.39	0.39
DL-Met	0.38	0.34	0.33	0.32	0.31
Na-bicarbonate	0.29	0.30	0.31	0.32	0.33
L-Thr	0.22	0.19	0.19	0.19	0.19
NaCl	0.16	0.15	0.14	0.14	0.13
L-Val	0.14	0.13	0.13	0.13	0.13
L-Arg	0.10	0.10	0.11	0.11	0.12
Phytase enzyme[2]	0.10	0.10	0.10	0.10	0.10
Coccidiostat	0.05	0.05	0.05	0.05	0.05
L-Ile	0.04	0.05	0.05	0.05	0.05
NSP-degrading enzyme[3]	0.01	0.01	0.01	0.01	0.01
Calculated nutrients, g/kg					
CP	205.0	195.0	187.8	183.3	177.0
ME, MCal/kg	2.73	2.82	2.88	2.91	2.96
Crude fat	42.19	55.00	62.06	66.48	72.66
Crude fiber	32.15	31.07	30.57	30.25	29.81
Crude ash	41.23	39.51	38.27	37.50	36.41
Total Ca	7.5	7.25	6.95	6.76	6.50
Total P	4.69	4.27	4.09	3.98	3.82
Total Na	1.5	1.50	1.50	1.50	1.50
Total Cl	2.2	2.1	2.06	2.04	2.00
Calculated digestible AA, g/kg					
dig. Lys	11.50	10.80	10.40	10.15	9.80
dig. Thr	7.82	7.24	6.97	6.80	6.57
dig. Met	6.04	5.61	5.39	5.25	5.05
dig. Met + Cys	8.63	8.10	7.80	7.61	7.35
dig. Trp	2.15	2.05	1.96	1.90	1.82
dig. Val	8.97	8.42	8.11	7.91	7.64
dig. Ile	7.48	7.13	6.83	6.64	6.37
dig. Leu	12.37	11.71	11.21	10.89	10.45
dig. Arg	11.85	11.12	10.71	10.45	10.09
dig. Phe	8.44	8.02	7.67	7.45	7.15
dig. His	4.17	3.95	3.77	3.66	3.51

CP = crude protein; ME = metabolizable energy; NSP = non-starch polysaccharide; AA = amino acid.

[1] Provided the following per kilogram of complete feed: vitamin A (retinyl acetate), 10,000 IU; cholecalciferol, 75 μg; vitamin E (all-rac-alfa-tocopheryl acetate), 50 IU; 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} (cyanocobalamine), 0.025 mg; niacinamide, 30 mg; folic acid, 1 mg; biotin/D-(+)-biotin, 0.15 mg; choline chloride, 689.7 mg; iron (II) sulphate (monohydrate) – iron, 49.2 mg; copper (II) sulphate (pentahydrate) – copper, 20 mg; zinc oxide, 60 mg; manganese (II) oxide – manganese, 95.9 mg; calcium iodate (anhydrous) – iodine, 1.2 mg; sodium selenite – selenium, 0.36 mg; sepiolite, 7 mg; propyl gallate, 2 mg; butylated hydroxytoluene, 3 mg; citric acid, 0,69 mg.

[2] Ronozyme HiPhos. Supplied the following per kilogram of complete feed: 1,000 FYT.

[3] Endo-1,4-β-xylanase (Ronozyme WX 2000 (CT)). Supplied the following per kilogram of complete feed: 200 FXU.

stakeholders). The starter diet was fed in mash form and the grower and finisher diets were pelleted using a 3-mm die. Both treatments received the same Starter diet. To create the rest of the feeding phases, 2 basal diets, Grower 1 and Finisher 2, were made. These 2 diets were mixed in a ratio of 0.6 to 0.4 to create Grower 2, and a ratio of 0.35 to 0.65 to create Finisher 1. All diets contained a phytase and non-starch polysaccharide degrading enzymes as well as a coccidiostat. The dietary treatments were analyzed for moisture concentration (Directive 71/393/EEC, 1971), CP (ISO 5983-2, 2009), crude fiber (AOCS Approves Procedure Ba 6a-05, 2005), crude fat (ISO 6492, 1999), and crude ash (ISO 5984, 2002) concentrations.

2.4. Ammonia concentrations at litter level

The NH_3 concentrations volatilized directly from the litter surface were measured at 23, 31, and 37 d of age by setting up a litter-level ammonia concentration measurement system. A Quantum Cascade Laser analyzer from Emerson (CT5200, Cascade Technologies, Stirling, Scotland) was coupled to an 8-channel multi sampler. Each channel of the multi-sampler was connected to a lid with a Teflon tube with a length of 50 m. Each lid had a bottom diameter of 52.5 cm, a rim height of 3 cm, and a height of 14 cm in the center. To measure the NH_3 concentrations, 3 sampling points per pen were identified, i.e. the center as well as the left and right sides at the back of each pen. A lid was placed on the litter surface at a sampling point and air was drawn through the tube to the analyzer at an air flow of 80 L/h. A measuring time of 5 min were allowed at each sampling point. From each of the 5-min measurements, a mean NH_3 gas concentration was calculated from the last 2 min of the steady state NH_3 gas concentration. Finally, a mean NH_3 concentration was calculated per pen and per treatment. To convert the ammonia concentration from ppm to milligram per cubic meter (mg/m^3), the following formula was used, assuming a temperature of 25 °C and pressure of 1 atmosphere:

$$NH_3 \text{ concentration } \left(mg \Big/ m^3\right) = 0.0409 \times NH_3 \text{ concentration (ppm)} \times \text{molecular weight of } NH_3 .$$

2.5. Litter scoring, sampling, and analysis

In this trial, litter was defined as the mixture of bedding material, bird excreta, and feathers. At 9, 17, 24, 32, and 39 d of age, the litter of each pen was evaluated and scored according to a 5-point scoring system of the Welfare Quality Assessment Protocol for poultry (2009): score 0 = completely dry and flaky, 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 together well, score 3 = sticks to boots and sticks readily in ball if compacted, score 4 = sticks to boots once the cap or compacted crust is broken.

Litter was sampled at 24, 32, and 39 d of age. In each pen, 5 different 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. From each sampling point, approximately 50 g of litter was collected. The litter of each pen was pooled, freeze-dried, ground to a size of 3 mm, and stored until analysis for moisture (Directive 71/393/EEC, 1971), total N (ISO 5983-2, 2009), total ammonia nitrogen (TAN) (BAM/deel 3/05, 2015), and uric acid concentrations (Marquardt, 1983).

The litter temperature and pH were measured on the same days. The litter temperature was measured from the same 5 sampling locations in each pen by inserting the probe of a digital thermometer (testo 110, Alton Hampshire, UK) directly underneath the litter surface. Subsequently, an average litter temperature was calculated per pen and per treatment. The pH of the litter was measured from 3 sampling points per pen: 2 points at the back (away from the feeders and drinkers) and 1 point in the center (in the proximity of the feeders and drinkers). At each sampling point, 20 g of litter was collected, mixed with 100 mL of distilled water and, after a waiting period of 30 min, the probe of a portable pH meter was inserted into the litter mixture to measure the pH. An average litter pH was calculated per pen and per treatment.

2.6. Foot- and hock lesion scores

At 9, 17, 24, 32, and 39 d of age, 16 birds per pen were randomly selected and the feet and hocks inspected. Foot- and hock lesions were scored according to a 5-point scoring system of the Welfare Quality Assessment Protocol for poultry (2009): score 0 = no lesions; score 1 and 2 = minimal evidence of lesions; score 3 and 4 = evidence of lesions.

2.7. Nitrogen balance

The method for the determination of whole-body N concentration was based on the procedures described by Bregendahl et al., (2002). In short, on d 9 of the trial, 6 chicks were randomly selected (3 chicks per treatment), fasted for 24 h (with free access to water), and euthanized by an intravenous overdose of sodium pentobarbital 20% (Kela NV, Hoogstraten, Belgium). Each carcass was ground, homogenized, and analyzed for the N concentration of the body (ISO 5983-2, 2009). The N concentration of these 6 carcasses were averaged and used as the baseline N concentration of the body. On d 39 of the trial, 6 birds per treatment were randomly selected, fasted for 24 h (with free access to water), and euthanized by an intravenous overdose of sodium pentobarbital 20% (Kela NV, Hoogstraten, Belgium). The carcasses were stored in plastic bags at −20 °C until further processing and analysis. The individual carcasses were autoclaved for 8 h, left to cool to room temperature and blended until a homogenized mixture was obtained. Representative samples were analyzed for N concentration (ISO 5983-2, 2009) to calculate the N concentration of the whole body at 39 d of age. The N retained was calculated as the difference between the N concentration of the body at 39 d of age and the baseline N concentration of the body at 9 d of age. The N excretion was calculated as the difference between the N intake from 9 to 39 d of age and the N retained. Furthermore, the N excretion and N retention as a percentage of the N intake, the N excretion as a percentage of the N retained, and the N excretion and N retention per kilogram BW were calculated.

2.8. Crude protein digestibility

At 31 d of age, 24 birds (2 birds per pen) were randomly selected and relocated to digestibility units. There were 12 digestibility units in total, and each unit housed 2 birds, resulting in 6 replications per treatment. Each digestibility unit consisted of a wire bottom with a plastic tray underneath for the collection of excreta and was equipped with a feed trough and drinker. The digestibility trial was performed according to the EU reference method (Bourdillon et al., 1990) and consisted of a 4-d adaptation period (from 31 to 34 d of age) and a 5-d balance period (from 35 to 39 d of age). During the balance period, the feed intake and total amount excreta were measured. The total feed intake was determined as the difference between the amount supplied and the amount refused per unit. After 2 d and at the end of the balance period the excreta of each unit were collected, mixed well, and a subsample per unit was freeze dried, ground to a size of 1 mm and stored for the analysis of CP (ISO 5983-2, 2009), uric acid (Marquardt, 1983), and moisture

concentration (Directive 71/393/EEC, 1971). The apparent dietary N digestibility coefficients were calculated correcting for uric acid N as follows:

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

The N retention for the digestibility study was calculated as follows:

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

After the balance period (at 39 d of age), the birds received the finisher treatments containing 0.4% titanium dioxide as an indigestible marker for 3 d. At 42 d of age, the birds were euthanized by an intravenous overdose of sodium pentobarbital 20% (Kela NV, Hoogstraten, Belgium) and ileal digesta samples were collected, pooled per unit, freeze-dried, ground to a size of 1 mm, and stored until further analysis for CP (ISO 5983-2, 2009) and titanium dioxide (Myers et al., 2004) to calculate the apparent ileal digestibility (AID) of CP (Ten Doeschate et al., 1993).

2.9. Performance

Pen weights were measured at the start of the trial (at 0 d of age) as well as at 9, 17, 24, 32, and 39 d of age. On the same days, feed consumption was recorded per pen to calculate the ADFI, BW, ADG, and FCR (after correcting for mortalities) for each period.

2.10. Carcass and meat yield and quality

Three days after the last weighing moment, at 42 d of age, 30 birds per treatment were randomly selected, individually marked, weighed, and fasted overnight. The fasted birds were transported to a commercial slaughterhouse where they were slaughtered and processed. The whole carcasses were transported back to the research facilities of ILVO where they were stored at 4 °C until the following day for the determination of carcass yield as well as the different meat cut yields (breast, thigh, drumstick, and wings). Carcass yield was calculated as eviscerated carcass weight relative to live weight before slaughter. The breast, thigh, drumstick, and wing yields were calculated weight relative to the eviscerated carcass weight. Different meat quality parameters were also determined: ultimate pH (pHu), temperature, meat color, drip loss, thawing loss, cooking loss, and shear force. The left breast muscle of each carcass was used for measuring temperature, pHu, meat color and drip loss. The temperature and pHu were measured by inserting the probe of a portable pH meter (Type HI98163 pH meter, Hannah Instruments, electrode FC2323, Woonsocket, Rhode Island, USA) into the breast meat. The color of the meat was evaluated for L* (lightness), a* (redness), and b* (yellowness) with a spectrophotometer (Hunterlab MiniScan, Reston, Virginia, USA). Three pHu and color measurements were taken from each left breast muscle and then averaged. After the color and pH measurements, each left breast muscle was sampled at 2 positions to determine the drip loss after 24 h at 4 °C according to 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 until the measurement of thawing loss, cooking loss, and shear force. After thawing the breast muscle samples overnight at room temperature, the thawing loss percentage was calculated as thawed weight relative to fresh weight. The thawed breast muscles were then cooked in a water bath for 45 min at 80 °C, cooled to room temperature, and weighed again to calculate cooking loss as a percentage of the thawed weight. The cooked breast muscles were stored in plastic bags at 4 °C overnight. 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 these sub-samples were measured using a Texture Analyzer (TA500, Lloyd Instruments, West Sussex, UK) fitted with a triangular Warner-Bratzler shear. For each breast muscle the highest and lowest shear force values were eliminated and the mean shear force was calculated from the remaining 8 values.

2.11. Data analysis

A randomized block design was used with in total 2 blocks of 6 pens. 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 the effect of dietary treatment as the main effect and block (location in the house) was included as a random effect. The experimental unit for performance, litter, and NH_3 concentration parameters was pen. For foot- and hock lesion scores as well as meat yield and quality parameters, and N balance parameters, the experimental unit was bird. For the digestibility parameters, the experimental unit was the digestibility unit. Results were expressed as least squares means and standard error of those means. Significant differences between least squares mean values of treatments were declared at a significance level of 5%. Normality of all data were evaluated through visual inspection of 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. Dietary treatments

The analyzed nutrient concentrations of the diets from the different phases were generally in good accordance with the formulated values (Table 2). The analyzed dietary CP concentration was higher than formulated values, but within the expected ranges, and still resulted in the anticipated differences in CP concentration between the 3-phase and 5-phase treatments.

3.2. Ammonia concentrations, litter scores, and litter composition

Compared to the 3-phase feeding program, the treatment consisting of 5-phases resulted in 37.9% and 20.8% lower NH_3 concentrations at litter level at 23 and 37 d of age, respectively ($P = 0.014$ and 0.028, respectively). Although no significant difference in NH_3 concentrations were measured at 31 d of age, the NH_3 concentrations were 13.2% lower for the 5-phase treatment than the 3-phase treatment ($P = 0.144$) (Table 3).

At 9 and 17 d of age all pens received a litter score of 0, and at 24 d of age, half of the pens from both treatment groups received a litter score of 1 (data not shown). The litter quality scores at 32 and 39 d of age are presented in Fig. 1A and B, respectively. There was no effect of the treatment on the litter scores at 32 and 39 d of age ($P = 0.213$ and $P = 0.721$, respectively).

Table 2
Analyzed nutrient concentrations of the 3-phase and 5-phase treatments.

Item	Starter	Grower 1	Grower 2	Finisher 1	Finisher 2
	0 to 9 d	9 to 17 d	17 to 24 d	24 to 32 d	32 to 39 d
Analyzed nutrients, g/kg					
Crude protein	212.2	203.7	193.3	185.2	177.4
Crude fat	44.8	57.9	64.3	67.0	71.2
Crude fiber	35.5	32.4	31.8	33.8	36.7
Crude ash	49.7	46.8	43.8	45.1	44.2

Table 3
Average ammonia concentrations measured at 23, 31, and 37 d of age from litter when feeding broiler chickens with 3 or 5 phases.

Item	Treatment		SEM	*P*-value
	3-phase	5-phase		
NH_3, mg/m^3				
d 23	13.58[a]	8.43[b]	2.24	0.014
d 31	41.65	36.15	2.65	0.144
d 37	51.89[a]	41.10[b]	3.47	0.028

SEM = standard error of the mean, NH_3 = ammonia.
[a, b] Treatments with different superscripts differ significantly for treatment ($P < 0.05$).

The litter analysis results are presented in Table 4. The dietary treatments had no effect on the TAN, total N, moisture, and uric acid content of the litter at 24, 32, and 39 d of age. The litter TAN content tended to be lower for the 5-phase treatment than the 3-phase treatment at 24 and 39 d of age ($P = 0.096$ and $P = 0.075$, respectively). The total litter N content also tended to be lower for the 5-phase treatment at 39 d of age ($P = 0.063$). The average litter temperature did not differ significantly between the treatments at 24, 32, and 39 d of age ($P = 0.405$, $P = 0.591$, and $P = 0.323$, respectively). At 24 and 32 d of age, the average litter pH was affected by the treatment ($P = 0.037$ and $P = 0.032$, respectively). The litter pH was lower for the 5-phase treatment than the 3-phase treatment at both 24 and 32 d of age. At 39 d of age, the litter pH was not significantly different between the treatments ($P = 0.290$).

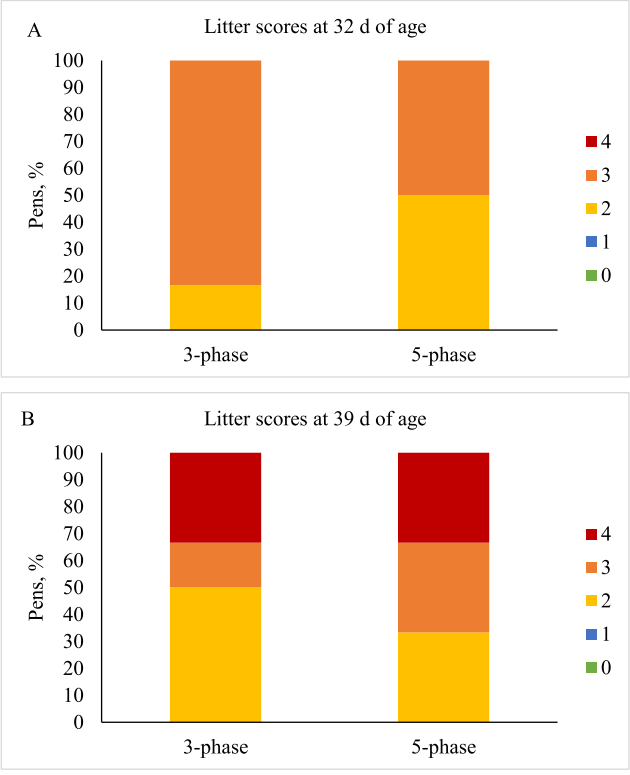

Fig. 1. Percentage of pens with different litter scores for the treatment groups: (A) 32 d of age, (B) d 39 d of age. Scoring: 0, completely dry and flaky; 1, dry, but not easy to move with foot; 2, leaves imprint of foot and will form a ball if compacted, but ball does not stay together well; 3, sticks to boots and sticks readily in a ball if compacted; 4, sticks to boots once to cap or compacted crust is broken (Welfare Quality Assessment protocol for poultry, 2009).

Table 4
Litter analysis at 24, 32, and 39 d of age from litter when feeding broiler chickens with 3 or 5 phases.

Item	Treatment		SEM	*P*-value
	3-phase	5-phase		
24 d of age				
TAN[1], mg/g	1.56	1.34	0.09	0.096
Total N[1], %	3.38	3.30	0.06	0.402
Moisture, %	43.6	42.5	1.2	0.521
Uric acid[1], %	2.90	2.91	0.11	0.959
Temperature, °C	27.8	27.6	0.2	0.405
pH	8.04	7.79	0.09	0.037
32 d of age				
TAN[1], mg/g	2.20	1.97	0.16	0.309
Total N[1], %	3.82	3.76	0.05	0.325
Moisture, %	47.0	45.6	0.8	0.223
Uric acid[1], %	3.26	3.00	0.11	0.103
Temperature, °C	28.4	28.5	0.2	0.591
pH	8.04	7.76	0.09	0.032
39 d of age				
TAN[1], mg/g	3.98	3.57	0.16	0.075
Total N[1], %	4.19	4.06	0.05	0.063
Moisture, %	44.8	44.3	1.2	0.754
Uric acid[1], %	3.33	3.26	0.15	0.706
Temperature, °C	28.5	28.3	0.2	0.323
pH	8.17	7.99	0.12	0.290

SEM = standard error of the mean, TAN = total ammoniacal nitrogen, N = nitrogen.
[1] Concentrations are reported as a percentage of the dry matter.

3.3. Foot- and hock lesion scores

No foot- and hock lesions were present at 9 d of age, and at 17 d of age the prevalence of foot- and hock lesions were negligible (data not shown). The foot- and hock lesion scores at 24, 32, and 39 d of age are presented in Fig. 2A–C, respectively. At 24 d of age, birds from the 3-phase treatment group had a higher prevalence of foot lesions than birds from the 5-phase treatment group ($P < 0.001$) and no differences were observed for hock lesions ($P = 0.500$). At 32 and 39 d of age, there was no significant difference in the prevalence of foot lesions ($P = 0.269$ and $P = 0.075$, respectively) and hock lesions ($P = 0.640$ and $P = 0.936$, respectively) between birds from the 3- and 5 phase treatment groups.

3.4. Crude protein digestibility

The results from the digestibility study are presented in Table 5. The dietary treatment had no effect on the apparent N digestibility corrected for uric acid N (determined by total collection of the excreta) or on the apparent ileal digestibility of CP ($P = 0.373$ and $P = 0.664$, respectively). The N retention tended to be lower for the 5-phase treatment than for the 3-phase treatment ($P = 0.052$).

3.5. Nitrogen balance

The results of the N balance study are summarized in Table 6. No significant differences between the treatments were detected for the different parameters. The N retention between 9 and 39 d was 7% higher for the 5-phase treatment than the 3-phase treatment

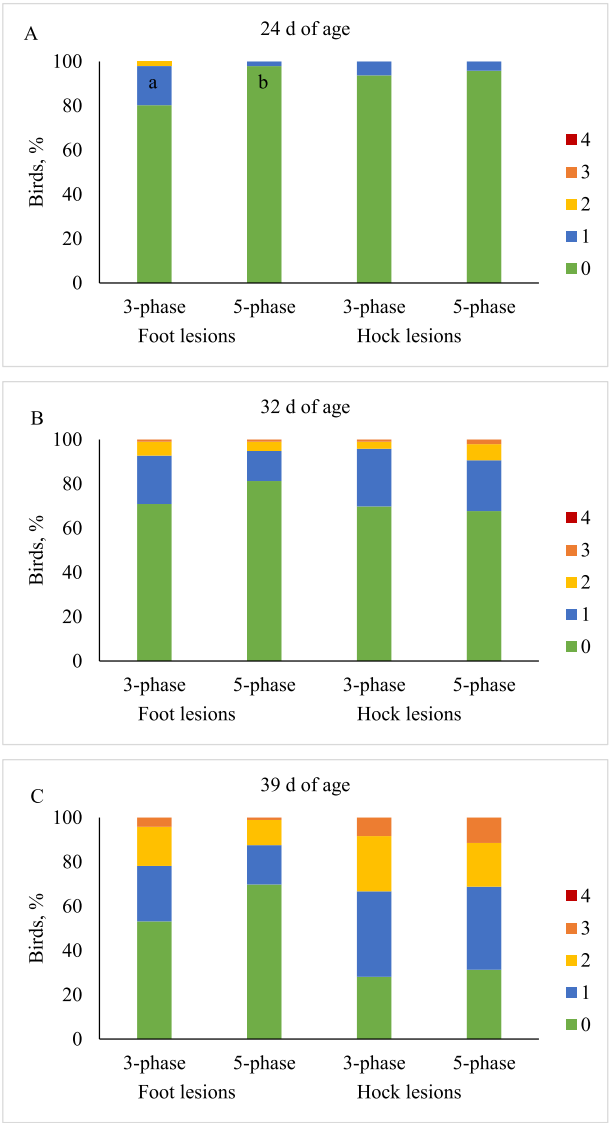

Fig. 2. Percentage of birds with different foot- and hock lesion scores at (A) 24 d of age, (B) 32 d of age, and (C) 39 d of age for the 3- and 5-phase treatment groups. [a, b] Treatments with different letters differ significantly for treatment ($P < 0.05$). Scoring: 0, no evidence of foot or hock lesions; 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).

($P = 0.423$). The apparent N excretion for the same period was 13% lower for the 5-phase treatment than the 3-phase treatment ($P = 0.232$).

3.6. Performance

The results of the different performance parameters measured for each phase as well as the overall period are presented in Table 7. No difference in performance was observed between the 3- and 5-phase treatment groups, except for the FCR between 17 and 24 d of age and for the overall period. For both these periods, birds from the 5-phase treatment had a higher FCR than birds from the 3-phase treatment ($P = 0.010$ and $P < 0.001$, respectively). The ADG between 17 and 24 d of age tended to be lower for the 5-phase treatment than the 3-phase treatment ($P = 0.081$).

Table 5
Apparent protein digestibility in broilers fed 3-phase and 5-phase diets.

Item	Treatment		SEM	*P*-value
	3-phase	5-phase		
Apparent N digestibility corrected for uric acid N[1], %	77.2	75.7	1.2	0.373
N retention, g	19.4	18.0	0.4	0.052
Apparent ileal digestibility of CP, %	76.9	77.8	1.4	0.664

SEM = standard error of the mean; N = nitrogen; CP = crude protein.
[1] Determined by total collection of the excreta.

Table 6
Body N content, N retention, and apparent N excretion of broilers fed 3- and 5-phase diets between 9 and 39 d of age.

Item	Treatment		SEM	*P*-value
	3-phase	5-phase		
Body N concentration at 39 d of age, %	2.90	2.92	0.25	0.625
N retention at 39 d of age, g	59.8	63.8	3.4	0.423
N excretion, g	45.8	39.7	3.4	0.232
N excretion/N intake, %	43.4	38.4	3.2	0.297
N retention/N intake, %	56.6	61.6	3.2	0.297
N excretion/N retention, %	81.5	63.3	10.8	0.261
N retention/BW, g/kg	27.0	26.9	0.4	0.876
N excretion/BW, g/kg	21.9	17.1	2.8	0.266

SEM = standard error of the mean, N = nitrogen.

Table 7
Productive performance of broilers fed 3- and 5-phase diets from 0 to 39 d of age.

Item	Period	Treatment		SEM	*P*-value
		3-phase	5-phase		
ADFI, g/bird per day	0 to 9 d	18.2	18.1	0.5	0.832
	9 to 17 d	54.9	56.0	2.5	0.405
	17 to 24 d	101.0	100.0	2.4	0.554
	24 to 32 d	139.0	140.0	2.5	0.729
	32 to 39 d	169.0	172.0	2.2	0.258
	0 to 39 d	92.6	93.3	2.2	0.258
BW, g/bird	9 d	196	193	7	0.546
	17 d	549	551	17	0.806
	24 d	1,092	1,077	22	0.418
	32 d	1,810	1,801	37	0.791
	39 d	2,549	2,535	40	0.686
ADG, g/bird per day	0 to 9 d	16.7	16.3	0.8	0.540
	9 to 17 d	44.2	44.8	1.3	0.367
	17 to 24 d	77.6	75.2	1.0	0.081
	24 to 32 d	89.7	90.5	1.9	0.726
	32 to 39 d	106	105	1.4	0.684
	0 to 39 d	64.2	63.8	1.0	0.684
FCR, g/g	0 to 9 d	1.10	1.11	0.05	0.845
	9 to 17 d	1.24	1.25	0.02	0.755
	17 to 24 d	1.30[a]	1.33[b]	0.02	0.010
	24 to 32 d	1.55	1.55	0.01	0.850
	32 to 39 d	1.61	1.64	0.01	0.130
	0 to 39 d	1.44[a]	1.46[b]	0.01	<0.001

SEM = standard error of the mean; ADFI = average daily feed intake; BW = body weight; ADG = average daily gain; FCR = feed conversion ratio.
[a, b] Means within a row with different superscripts differ significantly ($P < 0.05$).

3.7. Carcass and meat cut yield and quality

Values for live weight before slaughter, carcass yield, and the different meat cut yields are presented in Table 8. The dietary treatments had no effect on these parameters. Birds from the 3-phase treatment group were on average 124 g heavier before slaughter than birds from the 5-phase treatment group ($P = 0.126$), but both treatment groups had a carcass yield of 64.2%.

Table 8
Meat yield parameters of broilers fed 3- and 5-phase diets (%).

Item	Treatment		SEM	*P*-value
	3-phase	5-phase		
Live weight before slaughter, g/bird	3,052	2,928	58	0.126
Carcass yield	64.2	64.2	2.7	0.986
Breast yield	30.6	31.1	0.4	0.482
Drumstick yield	14.1	13.7	0.2	0.108
Thigh yield	28.1	27.6	1.2	0.089
Wings yield	10.3	10.4	0.1	0.591
Waste	16.6	17.0	0.2	0.273

SEM = standard error of the mean.

Table 9
Meat quality parameters of broilers fed 3- and 5-phase diets.

Item	Treatment		SEM	*P*-value
	3-phase	5-phase		
pHu	6.19	6.19	0.02	0.797
L*	57.8	57.0	0.5	0.196
a*	5.4	5.8	0.2	0.108
b*	14.8	14.8	0.4	0.859
Thawing loss, %	5.5	5.4	0.5	0.896
Cooking loss, %	23.2	23.5	0.7	0.647
Drip loss, %	1.5	1.2	0.3	0.324
Shear force, N	8.0	8.2	0.3	0.602

SEM = standard error of the mean; pHu = ultimate pH; L* = lightness; a* = redness; b* = yellowness.

The results of the different meat quality parameters are summarized in Table 9. These parameters did not differ significantly between the 3- and 5-phase treatment groups.

4. Discussion

Five-phase feeding compared with 3-phase feeding showed an important reduction of up to 37.95% in NH_3 volatilization from the litter on d 23. Although only numerically, this coincided with a lower TAN concentration in the litter itself, which likely exerted the lower litter pH. This lower litter pH in itself will have raised the fraction of ammonium (NH_4^+) within the TAN, hence decreasing the amount of ammonia which could be volatilized from the litter (Srinath and Loehr, 1974). The lower NH_3 volatilization in the 5-phase treatment may therefore be the result of a combination of lower litter pH and TAN concentration.

Given the reduction in protein intake while maintaining essential amino acid intake, it was logical to assume a reduced uric acid excretion, hence litter ammonia concentration, especially since no differences in digestibility were observed. Although numerical decreases in TAN as well as uric acid concentrations were measured, the proportional difference in litter TAN concentrations between the dietary treatments was far lower (e.g. −10.31% at d 39) than that of the ammonia volatilization (e.g. −20.81% at d 37). This further supports our conclusion that not only excretion but also the pH drop affected ammonia emission.

The total N and uric acid concentrations in the litter may partly be explained by the results obtained from the N balance study. When assessing the results from Table 6, it should be taken into account that only a small number of animals per treatment (6 birds per treatment) were used for the N balance study and therefore the BW of the selected birds may not exactly mirror the BW measured of all birds from the different treatments at 39 d of age (Table 7). The small sample size and increased variability reduced the power to detect significant effects between treatments. Although no significant differences between the 2 treatments were detected, the numerical differences are in favor of the 5-phase treatment. Bregendahl et al., (2002) found N excretion to be highly linearly correlated to N intake. In our study, a 2% lower cumulative N intake between 9 and 39 d of age for the 5-phase treatment than the 3-phase treatment resulted in a 6.7% higher N retention and 13.3% lower N excretion for the 5-phase treatment relative to the 3-phase treatment. Although not measured in this study, the higher N retention and lower N excretion for the 5-phase treatment were probably also maintained between 39 and 42 d of age given the fact that broilers from the 3-phase treatment also had a numerically higher BW than broilers from the 5-phase treatment before slaughter (at 42 d of age).

After the last weighing moment to determine BW of the birds at 39 d of age, the birds were kept until slaughter age (42 d of age), while receiving their respective dietary treatments. This explains the heavier BW before slaughter (Table 8) compared to the BW determined at 39 d of age (Table 6). The similar carcass and meat cut yields in this study agree with the literature (Warren and Emmert, 2000; Pope et al., 2004; Hauschild et al., 2015), however, Hauschild et al., (2015) did report a 3% higher breast meat yield in broilers fed a multiphase feeding program compared to broilers fed a 4-phase feeding program. Regarding the effect of phase feeding on performance parameters, the literature is inconclusive as some studies observed that phase feeding may improve growth during 5 and 6 wks of age (Gutierrez et al., 2008) or overall (Hauschild et al., 2015), while others reported no effect (Warren and Emmert, 2000; Pope and Emmert, 2002; Pope et al., 2004). Although there was no effect on carcass yield, the different meat cut yields, as well as ADFI, BW, and ADG, in our study, the FCR was significantly higher for the 5-phase treatment between 17 and 24 d of age as well as for the overall period. As the feed consumption during this period was similar for both treatments, the lower feed efficiency for the 5-phase treatment between 17 and 24 d of age results from a slightly lower growth during this period. Some studies comparing control and reduced CP diets in broilers have also reported a decrease in feed efficiency (Bregendahl et al., 2002; Hernandez et al., 2013). It should be kept in mind that feeding closer to the CP requirements of the animals also entails a higher risk of ending up below that requirement. The lower feed efficiency between 17 and 24 d of age and the as well as the numerically lower N retention observed during the digestibility study between 35 and 39 d of age suggests that during these 2 additional phases for the 5-phase treatment, there may have been a temporary deficit of a given essential amino acid, or an imbalanced ratio of essential amino acids to non-essential amino acids, reducing growth and feed efficiency. Both an over- and undersupply of essential amino acids, and an imbalanced ratio between the 2 could result in excess N being excreted as uric acid, and reducing N retention.

The reduced ammonia in the litter may have caused the lower incidence of foot lesions for birds from the 5-phase treatment than the 3-phase treatment at 24 d of age ($P < 0.001$). Other authors have also reported a decrease in the incidence of foot lesions with a decrease in the N and ammonia content of the litter (Shao et al., 2018; Lemme et al., 2019; Van Harn et al., 2019). Foot lesions in broilers are usually associated with wet litter (Shepherd and Fairchild, 2010; De Jong et al., 2014; Dunlop et al., 2016), although in this study, the litter moisture content at 24 d of age was not significantly different between the 2 treatments ($P = 0.521$). Furthermore, there were no significant differences between the 2 treatments for litter quality scores.

Our goal was to gradually decrease dietary CP and AA levels in accordance with the birds' requirements, to increase N retention and decrease N excretion per kg feed, and thereby decreasing NH_3 volatilized from the litter, while supporting performance and meat deposition. Our results are encouraging in that we found a

significant decrease in NH_3 volatilized from the litter. The absence of significant differences between the treatments in this study may be due to the fact that the CP and digestible amino acid levels were based on CVB (2016) standards as well as CP levels applied in practice in the EU, which are lower than NRC (1994) and breed (Aviagen, 2019) recommendations. If diets were formulated according to the latter recommendations, possibly larger differences in performance, and N retention and N excretion could have been observed. Despite no significant differences for N retention and excretion parameters, there was an indication of birds from the 5-phase treatment to have a higher N retention and lower N excretion, while maintaining performance and meat yield, although feed efficiency was slightly reduced. These results are promising as there is a possibility of lowering dietary N content by feeding more phases, while decreasing NH_3 emissions from commercial broiler facilities, which has several environmental advantages.

Author contributions

Madri Brink: Investigation, Visualization, Writing – original draft, Project administration, Conceptualization, Methodology. **Geert Janssens**: Supervision, Writing – review & editing. **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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