Effects of dietary crude protein levels on ammonia emission, litter and manure composition, N losses, and water intake in broiler breeders

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ABSTRACT This study determined the effects of different dietary crude protein (CP) levels on ammonia emission (NH_3), litter and manure composition, nitrogen (N) losses, and water intake in broiler breeders. A total of 480 females and 64 males (Ross 308) 20 wk of age were randomly allotted to 2 dietary treatments with 8 replicates of 30 females and 4 males per replicate. Birds were fed either high CP (CPh) or low CP diets (CPl) supplemented with free amino acids (AA). Both diets consisted of 3 sub-diets; 1 for each phase of the laying period. Diets were formulated to be isocaloric and calculated CP content of the CPl diets was 15 g/kg lower than the CPh diets (Breeder 1 (23 to 34 wk): 135 vs. 150, Breeder 2 (35 to 46 wk): 125 vs. 140 and Breeder 3 (47 to 60 wk of age): 115 vs. 130 g/kg, respectively). Pens consisted of an elevated slatted floor (25% of the floor surface) and a litter floor. Water and feed intake were recorded daily. Litter (floor) and manure (below slatted floor) composition and ammonia concentration were measured at 34, 44, and 54 wk of age. Ammonia concentration was measured using a flux chamber on top of the litter or manure. Estimated N losses were calculated. Dietary protein level did not affect water intake and dry matter (DM) content of the litter or manure. Compared to birds fed the CPh diets, the litter and manure samples of broiler breeders fed the CPl had 8% lower total-N and 13% lower ammonia-N content resulting in a 9% lower ammonia concentration, 9% lower ammonia emission, and 11% lower total-N losses. In conclusion, this study shows that reducing CP level in the diet of broiler breeders reduces ammonia emission and total N-losses from litter and manure.

Key words: broiler breeder, dietary crude protein, ammonia emission, litter and manure composition, N losses

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INTRODUCTION

Ammonia (NH_3) is a volatile nitrogen compound present in poultry houses from the microbial degradation of uric acid and undigested proteins from their diet in the droppings of the birds (Groot Koerkamp, 1994; Ferguson et al., 1998a). Inside poultry houses, concentrations of gaseous ammonia (above approx. 10 ppm) negatively affect the health, welfare, and productivity of the birds (Targowski et al., 1984; Kristensen and Wathes, 2000; Miles et al., 2004), as well as the health of workers (Donham and Cumro, 1999; Omland, 2002). Once emitted from the poultry houses, ammonia contributes to acidification, eutrophication, and reduced biodiversity in natural ecosystems (Lekkerkerk et al., 1995). Furthermore, ammonia reacts with other gases to form secondary particulate matter which contributes to air pollution as particles within the $PM_{2.5}$ fraction (Brunekreef et al., 2015). Therefore, enhancements are needed to reduce ammonia concentration within poultry houses and to lessen the nitrogen pollution of the poultry industry.

Commercial breeder layer diets often contain high levels of protein (150 to 160 g/kg) (de Beer, 2009) for growth rate and muscle deposition, hatching egg production, and protein turnover. This however, leads to a high N excretion (Lopez and Leeson, 1995a,b). In the last decades, several experiments have been carried out investigating effects of decreased dietary crude protein (**CP**) and/or amino acid (**AA**) levels on reproduction performance of breeders (e.g., Lopez and Leeson, 1995b; van Emous et al., 2013, 2015, 2018) without a negative effect on the reproductive performance of broiler breeder hens fed reduced CP diets (van Emous et al., 2018). Until now, however, no research has been carried out on the effect of low dietary CP on ammonia emission in breeders and only few papers (Lopez and Leeson, 1995a,b) have been published on the effect on N excretion of breeders. The latter authors carried out 3 experiments with 4 treatments consisting of diets with 20 to 14% CP (exp. 1), 15 to 9% CP (exp. 2) and 16 to 10% CP (exp. 3). N content of the excreta was reduced by 4 to 8% per 10 g/kg reduction of dietary CP level. More research on this topic has been done in the progeny of breeders (i.e., broilers) (Moran et al., 1992; Elwinger and Svensson, 1996;

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Ferguson et al., 1998a,b; Gates et al., 2000; Khajali and Moghaddam, 2006; Kamran et al., 2010; Ospina-Rojas et al., 2012; Hernandez et al., 2013; van Harn et al., 2017). These studies, in general, found a reduction of N excretion of 3 to 10% per 10 g/kg reduction of dietary CP level. Several studies with broilers showed a 10% reduction of ammonia emission per 10 g/kg reduction in dietary CP level (Elwinger and Svenson, 1996; Ferguson et al., 1998a,b; Ospina-Rojas et al., 2012; Hernandez et al., 2013). Furthermore, in broiler experiments, a decreased water intake due to a reduced CP intake has been found (Elwinger and Svensson, 1996; Hernandez et al., 2013; van Harn et al., 2017). This is caused by the decreased need to excrete the protein surplus from the body (in the form of uric acid). Furthermore, a lower water intake increases the DM content of the litter which in turn reduces the incidence of skin dermatitis like footpad lesions, hock burns, and breast blisters (van Harn et al., 2017). A reduced CP content in broiler breeder layer diets can be a feasible solution to reduce N losses in broiler breeder houses.

Due to current concerns about the effect of ammonia emission on the environment the development of strategies to reduce ammonia pollution from broiler breeders is necessary. Therefore, the current experiment was conducted to determine the effects of dietary CP levels in broiler breeder during the laying period on ammonia emission, litter and manure composition, N losses, and water intake in broiler breeders. Effects on reproduction performance from the present experiment have been reported in another paper (van Emous et al., 2018).

MATERIALS AND METHODS

Experimental Design

A completely randomized experimental design consisting of 2 dietary CP levels with 8 replicates of 30 females and 4 males per replicate was applied. The breeders received 1 of 2 diets with either a high CP level (**CPh)** or a low CP level (**CPl**) (Table 1) and both diets consisted of 3 sub diets; 1 for each phase of the laying period.

The CPh diets were formulated to be iso-caloric [2,800 kcal/kg nitrogen corrected apparent metabolize energy (**AME$_n$**)] based on the recommendations of the breeder company (Aviagen-EPI, 2015). The calculated CP content of the CPl diets was 15 g/kg lower than the CPh diets [Breeder 1 (22 to 34 wk): 135 vs. 150, Breeder 2 (35 to 46 wk): 125 vs. 140 and Breeder 3 (47 to 60 wk of age): 115 vs. 130 g/kg, respectively]. The diets were supplemented with free AA (Lys, Met, Thr, Trp, Arg, Ile, and Val) to meet the minimum essential AA levels recommended by the breeder company (Aviagen-EPI, 2015). Birds of both diets were fed the same daily amount of feed to provide a different daily nutrient (particularly CP and AA) intake (more details: van Emous et al., 2018). Males received a standard male diet (2,600 AME$_n$ kcal/kg; 13.0% CP; 0.45% dig. Lys; 0.5% dig. M+C; 1.0% Ca; 0.3% aP). During the first 3 wk birds received a standard pre-breeder diet according to the recommendation of the breeder company (Aviagen-EPI, 2015).

Birds, Housing, and Management

A flock of 480 female and 64 male Ross 308 broiler breeders at 20 wk of age (PoultryPlus, Ambt Delden, The Netherlands) were allotted to 16 floor pens with 34 birds per pen (30 females and 4 males). Pens were located in 4 separated experimental rooms (4 pens per room; both treatments were applied in each room). Each room was mechanically ventilated to remove the heat, moisture, carbon dioxide, and ammonia from the birds and ensure proper climate. Pens measured 1.8 m × 4.0 m (7.2 m^2; 4.7 birds/m^2). They consisted of a litter floor (75% of the floor surface; 2 kg/m^2 of wood shavings as bedding material) and an elevated floor with plastic slats (1.8 × 1.0 m = 1.8 m^2; 25% of the floor surface). Each pen was equipped with 4 nests boxes (94 × 33 cm) and were available to the breeders from 23 wk age onwards. Water was provided ad libitum via 7 drink cups above the slats. Females were fed in 2 feeding troughs with a male exclusion system (3 m each) at the walls of the pens. Males were fed in 1 feeding pan positioned at a height of 50 cm to prevent female access. Birds were maintained on the same target BW and feed allocation was adjusted to the predetermined body growth curve during rearing and a combination of the predetermined body growth curve and egg production (Aviagen-EPI, Roermond, The Netherlands). Breeders were photostimulated with 11 h of light (40 lx) at 21 or 23 wk of age. After photostimulation, day length was gradually increased by 1 h (later 0.5 h) per week to a photoperiod of 14L:10D. This was maintained until the end of the experiment at 60 wk of age, with lights on from 0330 to 1730 h (40 lx). Temperature was maintained at 20°C during the entire period by means of the mechanical ventilation.

The protocol for the experiment conformed to the standards for animal experiments and was approved by the Ethical Committee of Wageningen UR, the Netherlands (protocol number: 2016012).

Observations

Water and Feed Intake Water intake was measured daily by visual inspection of the scaling on the water bins. Water−feed ratio was calculated by dividing the daily water intake by the daily feed intake per pen. Daily feed rations were weight weekly, and birds fed the CPl diet received the same daily amount of feed than those fed the CPH.

Litter and Manure Composition At 34, 44, and 54 wk of age 4 litter sub samples and 2 manure sub samples (below slatted floor) per pen of approximately 200 g were taken from the full depth of the litter and manure layer (away from feeders and drink cups). Sub

Table 1. Dietary ingredients, and analyzed and calculated nutrients of the rearing diets (g/kg, as-fed basis).

Item	Pre breeder	Breeder 1 (23 to 34 wk)		Breeder 2 (35 to 46 wk)		Breeder 3 (47 to 60 wk)	
		CPh[1]	CPl	CPh	CPl	CPh	CPl
Ingredient							
Corn	683.6	650.0	665.7	660.0	694.4	685.2	719.1
Soybean meal	78.7	144.3	104.1	119.0	76.6	93.8	47.5
Sunflower meal	100.0	65.0	55.0	65.0	55.0	65.0	55.0
Wheat bran	96.0	36.5	69.6	45.0	65.0	45.0	68.6
Palm oil	2.0	1.2	–	8.0	1.1	7.3	–
Soya oil	–	15.1	14.5	9.5	9.8	7.1	7.4
Limestone	11.6	39.3	37.7	44.9	43.2	47.9	46.2
Chalk	10.0	30.0	32.0	30.0	32.0	30.0	32.0
Monocalcium phosphate	5.2	6.0	6.2	5.6	6.0	5.3	5.7
Salt	2.7	3.0	2.6	2.9	2.4	2.8	2.2
Sodium carbonate	2.7	2.3	2.9	2.4	3.1	2.6	3.4
Premix[2]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Curb	2.0	2.0	2.0	2.0	2.0	2.0	2.0
L-Lysine	0.7	0.1	1.4	0.4	1.8	0.8	2.3
DL-Methionine	0.8	1.2	1.6	1.2	1.6	1.1	1.5
L-Threonine	–	–	0.5	0.1	0.7	0.1	0.9
L-Tryptophan	–	–	–	–	0.1	–	0.2
L-Arginine	–	–	–	–	0.6	–	1.0
L-Isoleucine	–	–	0.2	–	0.5	–	0.7
L-Valine	–	–	–	–	0.1	–	0.3
Calculated content							
AME_n (kcal/kg)	2,800	2,800	2,800	2,800	2,800	2,800	2,800
CP	142.5	150.0	135.0	140.0	125.0	130.0	115.0
Dig. Lys	5.1	5.7	5.7	5.3	5.3	5.0	5.0
Dig. Met+Cys	5.1	5.5	5.5	5.2	5.2	4.9	4.9
Dig. Thr	4.2	4.6	4.5	4.3	4.3	4.0	4.0
Dig. Trp	1.4	1.5	1.3	1.4	1.2	1.3	1.2
Dig. Ile	4.8	5.4	4.8	4.9	4.6	4.5	4.3
Dig. Val	5.7	6.1	5.4	5.7	5.0	5.2	4.7
Dig. His	3.2	3.4	3.0	3.2	2.7	2.9	2.5
Dig. Phe	6.0	6.6	5.7	6.1	5.2	5.6	4.6
Dig. Gly	5.1	5.2	4.6	4.8	4.2	4.5	3.8
Dig. Ser	5.6	6.1	5.4	5.7	4.8	5.2	4.3
Na	1.8	1.8	1.8	1.8	1.8	1.8	1.8
K	7.0	7.0	6.4	6.6	5.9	6.1	5.3
Cl	2.3	2.3	2.3	2.3	2.3	2.3	2.3
DEB (mEq)	192	192	178	182	163	169	150
Calcium	12.0	30.0	30.0	32.0	32.0	33.0	33.0
Total phosphorus	5.3	4.8	4.8	4.6	4.6	4.5	4.5
Available phosphorus	3.2	3.2	3.2	3.1	3.1	3.0	3.0
Linoleic acid	15.7	22.0	22.0	20.0	20.0	19.0	19.0
Analyzed content							
CP	137.0	149.6	136.6	137.9	124.4	127.0	112.8
Lysine	6.33	6.78	6.82	6.16	6.17	6.04	5.93
Meth+Cyst	5.47	5.96	5.88	5.66	5.79	5.45	5.39
Threonine	4.99	5.51	5.42	5.18	5.12	4.81	4.77
Tryptophan	1.64	1.72	1.51	1.60	1.44	1.43	1.32
Arginine	9.01	9.90	8.76	9.00	8.09	8.28	7.50
Isoleucine	5.29	6.13	5.53	5.51	5.07	5.03	4.72
Leucine	11.71	13.23	11.94	12.09	10.76	11.43	9.98
Valine	6.60	7.22	6.45	6.59	5.78	6.14	5.47
Histidine	3.65	3.96	3.58	3.65	3.19	3.41	2.92
Phenylalanine	6.62	7.49	6.62	6.77	5.78	6.28	5.17
Glycine	6.46	6.65	5.95	6.12	5.30	5.75	4.86
Serine	6.52	7.23	6.37	6.48	5.61	6.04	5.00

[1]Dietary protein level. CPh = high dietary protein level rearing; CPl = low dietary protein level rearing.
[2]Provided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D3, 1,500 IU; vitamin E, 100 mg; vitamin K3, 3.0 mg; vitamin B1, 3.0 mg; vitamin B2, 10.0 mg; vitamin B6, 4.0 mg; vitamin B12, 0.03 mg; niacinamide, 30 mg; D-pantothenic acid, 16.3 mg; choline chloride, 344 mg; folic acid, 2.0 mg; biotin, 0.3 mg; iron, 100 mg; copper, 15 mg; manganese, 100 mg; zinc, 100 mg; iodine, 2.0 mg; selenium, 0.15 mg.

samples were taken from different locations within the pen to include spatial variation in the sample. Sub samples were first mixed and then 200 g of this mixed sample was stored at −20°C awaiting analysis. Litter and manure samples were analyzed for dry matter (**DM**) content by gravimetric determination after oven drying for 4 h at 103°C (±3°C) (NEN 7432, 1996). Ash was analyzed by gravimetric determination after drying

for 4 h at 550°C (±10°C) (NEN 7432, 1996). Total-N and ammonia-N (NH_4+) was analyzed by distillation (NEN 7433, 7434, and 7438, 1998). At the same days, the pH was measured at room temperature (approximately 20°C) with an Orion 9104SC.

Ammonia Concentration and Emission Ammonia concentration was measured at 34, 44, and 54 wk of age using a flux chamber placed on the litter or on the manure under the slatted floor. The flux chamber measured 60 × 40 × 15 cm (length × width × height; surface area: 0.24 m^2; volume: 0.036 m^3). On both sides of the flux chamber a tapered air duct guided the air over the emitting surface. Air entered and left the flux chamber through a round flexible tube with a diameter of 0.35 m (P-super, Panflex, Ede, the Netherlands). A ventilator (Fancom FMS 35; 3,000 m^3/h max. ; Fancom, Panningen, the Netherlands) with ventilation control unit (Fancom FCTA; Fancom, Panningen, the Netherlands) was used to pull the air through the flux chamber and over the emitting surface. Ventilation was set at a constant value of 30% of the maximum capacity (i.e., 900 m^3/h) resulting in an average air velocity across the emitting surface of 0.57 m/s. Incoming air was taken from outside the experimental facility to guarantee clean air. Ventilation rate was measured using a fan wheel anemometer placed upstream of the ventilator in the outgoing air flow. Both incoming and outgoing air were sampled using a PE sampling line. Concentration of ammonia in the incoming and outgoing air was measured using 2 photo-acoustic multi gas monitors (Innova 1312; LumaSense Technologies, Santa Clara, USA). Concentrations were measured during 30 min. During the last 15 min, concentrations of ammonia of both incoming and outgoing air were also measured by sucking an air sample (1,000 mL/min restricted flow) through 2 glass impingers placed in serial, each containing 100 mL of 0.5 M sulfuric acid solution. Halfway the 30 min measuring period, concentrations of ammonia were checked using gas detection tubes (Kitagawa; type No. 105SD 0.2 to 20 ppm; Kitagawa, Komyo Rikagaku Kogyo, Japan). The ammonia flux (Q; mg/h per m^2) from the emitting surface was calculated by equation (1):

$$Q = \frac{\phi\ (C_{in} - C_{out})}{A}, \qquad (1)$$

where C_{in} (mg/m^3) is the concentration of ammonia entering the flux chamber; C_{out} (mg/m^3) is the concentration of ammonia leaving the flux chamber; ϕ (m^3/h) is the ventilation rate; and A (m^2) is the emitting surface.

Estimate of Nitrogen (N) Losses Environmental total N losses per hen housed were calculated by equation (2):

$$N_{\text{total losses}} = N_{\text{litter start}} + N_{\text{intake female}} + N_{\text{intake male}} - N_{\text{eggs}} - N_{\text{female deposited}} - N_{\text{male deposited}} \qquad (2)$$

Environmental gaseous N losses per hen housed were calculated by equation (3):

$$N_{\text{gaseous losses}} = N_{\text{litter start}} + N_{\text{intake female}} + N_{\text{intake male}} - N_{\text{eggs}} - N_{\text{female deposited}} - N_{\text{male deposited}} - N_{\text{litter end}} - N_{\text{manure end}} \qquad (3)$$

where $N_{\text{litter start}}$ is the N present in the litter at the start of the experiment (assumed to be 6.8 g/kg; N'Dayegamiye and Isfan, 1991), $N_{\text{intake female}}$ is the N consumed by the females (calculated from the feed intake and the feed N content); $N_{\text{intake male}}$ is the N consumed by the males (calculated from the feed intake and the feed N content); N_{eggs} is the N deposited in the eggs, calculated from the total mass of eggs produced and the egg N content (the latter was assumed to be 19.3 g/kg; Jongbloed and Kemme, 2005); $N_{\text{female deposited}}$ = deposition of body N of the females from start until end of the experiment, including deposition in dead birds (assumed to be 33.4 and 31.5 g/kg live weight at 20 and 60 wk of age, respectively, independent of dietary CP level; Nonis and Gous, 2016); $N_{\text{male deposited}}$ = deposition of body N of the females from start until end of the experiment, including deposition in dead birds (assumed to be 34.5 and 35.4 g/kg live weight at 20 and 60 wk of age, respectively, independent of dietary CP level; Jongbloed and Kemme, 2005); $N_{\text{litter end}}$ = N present in the litter at the end of the experiment, calculated from the amount of litter present (calculated via the ash balance, see below) and the N content of the litter as measured at 54 wk of age; $N_{\text{manure end}}$ = N present in the manure at the end of the experiment, calculated from the amount of manure produced (calculated via the ash balance, see below) and the N content of the manure as measured at 54 wk of age. The quantity of litter and manure was estimated by calculating the ash balance. The total amount of ash in the litter and manure was calculated by subtracting the ash retention in the birds (ash retention per kg growth from Gous et al., 1999) and the amount of ash in eggs (ash content from and van den Brand et al., 2004 and Matt et al., 2009) from the ash intake in the feed plus the amount of ash in wood shavings (ash content from Alakangas, 2005). The amount of litter and manure was estimated under the assumption of a litter/manure ratio equal to 2 to 1 (van Emous, non-published data).

Statistical Analysis

The data were analyzed using Genstat statistical software (Genstat, 2015). Statistical significance was declared at $P < 0.05$, with $0.05 < P < 0.10$ considered as a tendency.

Response variables with regard to litter composition, manure composition, and ammonia emission were analyzed using the REML (REstricted Maximum

Likelihood) directive of GenStat according the following model:

$$Y_{ijkm} = \mu + R_i + CP_j + A_k + L_m + \varepsilon_{ijkm} \quad (4)$$

where Y_{ijkm} is the response variable, μ the overall mean, R_i the random effect of room ($i = 1...4$), CP_j the effect of CP diet (CPh, CPl; $j = 1, 2$), A_k the effect of age (34, 44, 54 wk of age; $k = 1...3$), L_m the effect of sampling location in the pen (litter, manure under slatted floor; $m = 1, 2$), and $\mathcal{E}_{ijkm}$ the residual error term. Pen was the experimental unit. Interaction effects between CP_j, A_k and L_m were tested for significance but excluded from the final model when not significant. Significant differences between levels of a factor were determined using t-tests.

Response variables with regard to feed and water intake, N balance and N loss were analyzed using Analysis of Variance (ANOVA) with room as block and CP diet (CPh, CPl) as treatment.

RESULTS AND DISCUSSION

Ammonia Concentration and Emission

The results on concentrations and emissions of ammonia as affected by dietary protein level are shown in Table 2. Ammonia concentration was 9.2% lower (4.76 vs. 5.24 ppm; $P = 0.039$), and ammonia emission was 9.0% lower (103.6 vs. 113.8 g/h per m^2; $P = 0.017$), in pens with birds fed the CPl diet in comparison to pens with birds fed the CPh diet. This reduction applied to both the litter and the manure under the slatted floor.

Table 2. Ammonia concentration and emission as affected by dietary protein level (CP) and age at sampling.

Item	NH_3 concentration (ppm)	NH_3 flux (mg/h per m^2)
CP level[1]		
CPh	5.24[a]	113.8[a]
CPl	4.76[b]	103.6[b]
SEM	0.160	4.90
P-value	0.039	0.017
Reduction, CPl vs. CPh	9.2%	9.0%
Location[2]		
Litter	5.19	111.8
Manure	4.81	105.7
SEM	0.155	4.90
P-value	0.06	0.15
Age		
34 wk	4.72[b]	116.8[a]
44 wk	4.51[b]	103.9[b]
54 wk	5.77[a]	105.4[b]
SEM	0.184	5.30
P-value	<0.001	0.028

[a,b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level; CPl = low dietary protein level.
[2]Flux chamber measurement above litter or above the manure under the slatted floor.

Table 3. Litter and manure composition as affected by dietary protein level (CP) and age at sampling.

Item	Total-N (g/kg DM)	Ammonia-N (g/kg DM)	DM (g/kg)	Ash (g/kg)	pH
CP level[1]					
CPh	36.2[a]	6.4[a]	556	181	7.6
CPl	33.2[b]	5.6[b]	568	190	7.6
SEM	0.58	0.21	14.6	3.3	0.06
P-value	<0.001	0.014	0.59	0.064	0.73
Location[2]					
Litter	36.5[a]	6.5[a]	601[a]	187	7.8[a]
Manure	32.8[b]	5.5[b]	523[b]	184	7.4[b]
SEM	0.58	0.21	12.5	3.3	0.06
P-value	<0.001	0.002	<0.001	0.55	<0.001
Age					
34 wk	35.5	8.7[a]	456[b]	153[b]	8.5[a]
44 wk	33.8	5.0[b]	609[a]	196[a]	7.1[b]
54 wk	34.7	4.3[b]	622[a]	207[a]	7.2[b]
SEM	0.71	0.25	14.4	4.0	0.07
P-value	0.23	<0.001	<0.001	<0.001	<0.001

[a,b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level; CPl = low dietary protein level.
[2]Litter = sample from the litter; manure = sample from the manure under the slatted floor.

Ammonia production and volatilization from litter and manure depends on temperature, pH, water availability and physical-chemical interactions in the litter and manure, but the level of N excreted from the birds as undigested proteins and uric acid to the litter and manure is the basis for differences in ammonia production (Groot Koerkamp, 1994; Fergusson et al., 1998a). The results from the present work further supports evidence from previous studies that reducing dietary CP level in broilers reduces ammonia concentration (Ferguson et al., 1998b; Gates et al., 2000; Ospina-Rojas et al., 2012; Hernandez et al., 2013) and emission (Elwinger and Svensson, 1996; Fergusson et al., 1998a). Several studies in broilers have shown an effect between dietary CP level and excretion of N (Moran et al., 1992; Elwinger and Svensson, 1996; Ferguson et al., 1998a,b; Khajali and Moghaddam, 2006; Kamran et al., 2010; Ospina-Rojas et al., 2012; van Harn et al., 2017).

The reduction of ammonia emission in the present study (6% per 10 g/kg lower dietary CP level) was lower than expected based on previous studies in broilers by Ferguson et al. (1998b), Ospina-Rojas et al. (2012), and Hernandez et al. (2013). These authors found reductions of ammonia emission in the range of 8 to 14% per 10 g/kg reduction of dietary CP level. An explanation for this could be that, in the present study, factors other than dietary CP (temperature, pH, and moisture content) were very similar between the diets in both litter and manure (Table 3).

Litter Composition

The results of litter and manure composition as affected by dietary CP level are shown in Table 3. Total-N was 8% lower (33.2 vs. 36.2 g/kg DM; $P < 0.001$) and ammonia-N was 13% lower (5.6 vs. 6.4 g/kg DM;

$P = 0.014$) in the litter and manure samples of the birds fed the CPl diet as compared to the birds fed the CPh diets. The dietary CP level did not affect the DM content ($P = 0.59$), ash content ($P = 0.064$), and pH ($P = 0.73$) of litter and manure.

In the present study with broiler breeders, total-N in the litter and manure was 6% lower per 10 g/kg reduction of dietary CP level. This result can be compared with those from experiments with their offspring, i.e., broilers (Moran et al., 1992; Elwinger and Svensson, 1996; Ferguson et al., 1998a,b; Khajali and Moghaddam, 2006; Kamran et al., 2010; Ospina-Rojas et al., 2012; van Harn et al., 2017). These studies showed a 3 to 10% lower total-N content in the litter per 10 g/kg reduction of dietary CP level.

Total-N content was 10% lower (32.8 vs. 36.5 g/kg DM; $P < 0.001$) and ammonia-N content was 14% lower (5.5 vs. 6.5 g/kg DM; $P = 0.002$) for the manure samples as compared to the litter samples (Table 3). This is surprising given the fact that litter is manure too which originated from the same birds. Apparently, the net result of all factors influencing microbial degradation of uric acid and undigested proteins to ammonia were more favorable in the manure than in the litter. These factors can be a higher water content in manure (477 vs. 399 g/kg), more aerobic conditions in the generally more crumbly litter, and possibly a higher temperature in the manure than in the litter due to composting.

The ash content of the litter and manure in the present study was not affected by dietary CP level (Table 3), which is in agreement with earlier studies in broilers (Moran et al., 1992; Kamran et al., 2010). Furthermore, the ash content did not differ between litter and manure which can be explained by the fact that equal amounts of feed (i.e., the main source of ash) were fed to all birds. The ash content of litter and manure increased with the age of the birds, most likely due to drying which is reflected in the increase in DM content with age (i.e., in time).

Research of Taraba et al. (1980) showed that, besides other factors, the pH of litter has a large influence on ammonia production. Normally, the pH of litter ranges between 6.5 and 8.5 (Anthony et al., 1994; Ferguson et al., 1998a; Khajali and Moghaddam, 2006, van Harn et al., 2017). A pH below 7.0 (neutral) reduces the uricolytic bacterial population responsible for ammonia production and increases the population of other bacteria which absorb ammonia, resulting in less ammonia volatilization to the environment (Ferguson et al., 1998a,b). In the present study, dietary CP level did not affect the pH of the litter and manure (Table 3). This finding is in agreement with those from broiler experiments by Ferguson et al. (1998a) and van Harn et al. (2017) but contrasts with those from broiler experiments by Ferguson et al. (1998b) and Khajali and Moghaddam (2006). The latter studies found a lower pH of litter when broilers were fed diets with lower CP levels. Thus, the literature is inconclusive on this matter. Possibly, the absence of an effect of dietary CP level on pH in the present study and previous ones (Ferguson et al., 1998a; van Harn et al., 2017) has been caused by the presence of cakes on the litter. Litter covered with such a top layer definitely shows different characteristics, however the literature is inconclusive on this matter.

The pH of the manure (under the slatted floor) was lower (7.4 vs. 7.8; $P < 0.001$) as compared to the litter on the floor (Table 3) which coincided with a lower ammonia content (5.5 vs. 6.5 g/kg; $P = 0.002$). This finding is consistent with prior studies of Carr et al. (1990) and Ferguson et al. (1998a,b). It is postulated by Elliot and Collins (1982) that especially the combination of moisture content and pH controls the release of ammonia from manure. Relative small changes in pH ($-\log[H^+]$) results in large changes in $[H^+]$ concentration which, in turn, largely affects the free (unionized) ammonia content of the manure. Over time, pH of the litter and manure samples decreased from 8.5 to higher than 7.0, reaching a stable situation from 44 wk of age onwards (Table 3). This is generally in agreement with the previous discussion about the correlation between pH and ammonia-N content in this paper.

Nitrogen Balance and Losses

Table 4 shows that birds fed the CPl diets showed a 11% lower loss of total N (644 vs. 727 g N per hen housed; $P < 0.001$) and a 14% lower loss of gaseous N (294 vs. 340 g N per hen housed; $P = 0.008$) as compared to the birds fed the CPh diet. Total N losses (N losses in the excretion) in the present study was 7% lower calculated for a 10 g/kg lower dietary CP level which is in agreement with previous studies with breeders and broilers. An 8% lower total N loss per

Table 4. N balance and N losses (g per hen housed) as affected by dietary protein level (CP).

Item	CPh[1]	CPl[1]	SEM	*P*-value
N_{input}				
$N_{litter\ start}$	2	2	–	–
$N_{intake\ female}$	910[a]	817[b]	5.0	<0.001
$N_{intake\ male}$	96	97	3.6	0.80
$N_{total\ input}$	1,008[a]	916[b]	4.6	<0.001
N_{output}				
N_{eggs}	234	229	2.3	0.12
$N_{female\ deposited}$	41	37	2.4	0.26
$N_{male\ deposited}$	6	7	0.6	0.65
$N_{litter\ end}$	246	226	8.3	0.10
$N_{manure\ end}$	140	125	7.6	0.17
$N_{total\ output}$	669[a]	623[b]	10.6	0.011
$N_{total\ losses}$[2]	727[a]	644[b]	3.9	<0.001
$N_{total\ losses\ (\%)}$[3]	72.1[a]	70.3[b]	0.40	0.005
$N_{gaseous\ losses}$[4]	340[a]	294[b]	10.0	0.008
$N_{gaseous\ losses\ (\%)}$[3]	33.7	32.0	1.10	0.28

[1]CPh = high dietary protein level; CPl = low dietary protein level.
[2]$N_{total\ losses} = N_{litter\ start} + N_{intake\ female} + N_{intake\ male} - N_{eggs} - N_{female\ deposited} - N_{male\ deposited}$.
[3]$N_{total\ losses\ (\%)}$ and $N_{gaseous\ losses\ (\%)}$ = expressed as percentage of $N_{total\ input}$.
[4]$N_{gaseous\ losses} = N_{litter\ start} + N_{intake\ female} + N_{intake\ male} - N_{eggs} - N_{female\ dep} - N_{male\ dep} - N_{litter\ end} - N_{manure\ end}$.

10 g/kg lower dietary CP level has been found in breeders (Lopez and Leeson, 1995a,b) and a 9% lower total N loss per 10 g/kg lower dietary CP level has been found in broilers (Elwinger and Svensson, 1996; Kamran et al., 2010; Hernandez et al., 2013). The difference between the CPh and CPl diets in calculated gaseous N losses was higher (14%) as compared to the reduction in ammonia emission (9%; Table 3). This may have been caused by underestimation of the N content of manure and litter because of the use of measured N content values of the manure and litter from 54 wk of age instead of 60 wk of age. Moreover, the total amount of litter and manure was calculated via the ash balance method which included assumptions (values from literature) on the ash content of eggs, birds, and bedding material.

Water and Feed Intake and Water/Feed Ratio

The results on water intake, feed intake, and water/feed ratio as affected by dietary CP level are shown in Table 5. Dietary CP level affected neither of the 3 variables. Studies in broilers, however, did find a lower water intake and water/feed ratio with lower dietary CP levels (Elwinger and Svensson, 1996; Bailey, 1999; Hernandez et al., 2013; van Harn et al., 2017). Elwinger and Svensson fed broilers diets with CP levels of 22 (control), 20 or 18% during the entire growth period. They found that water intake was 3.5 and 7.0% lower for the 20 and 18% CP diet as compared to the control (22%), respectively, whereas water/feed ratio was 2 and 5% lower. This finding is in agreement with research in broilers by van Harn et al. (2017) with 4 different treatment groups (control, −10, −20, and −30 g/kg CP). The latter authors found that water intake and water/feed ratio decreased linearly with dietary CP level. The lowering effect on water intake is caused by the lower protein intake which decreases the amount of metabolites that need to be excreted in urine: a process which enhances water intake (Elwinger and Svensson, 1996; van Harn et al., 2017).

Table 5. Water intake, feed intake and water/feed ratio as affected by dietary protein level (CP).[1]

Item	CPh	CPl	SEM	*P*-value
Feed intake	g/b/D			
Breeder 1	143.6	143.7	0.13	0.81
Breeder 2	158.6	156.2	0.36	0.09
Breeder 3	157.4	155.6	0.67	0.43
Overall	153.1	151.8	1.03	0.28
Water intake	mL/b/D			
Breeder 1	272.0	274.7	1.50	0.56
Breeder 2	295.7	288.2	1.75	0.26
Breeder 3	288.6	289.4	1.93	0.91
Overall	285.5	284.4	4.69	0.85
Water/feed ratio	mL/g			
Breeder 1	1.89	1.91	0.023	0.60
Breeder 2	1.87	1.85	0.023	0.57
Breeder 3	1.83	1.86	0.022	0.40
Overall	1.87	1.87	0.020	0.75

[1]CPh = high dietary protein level; CPl = low dietary protein level.

CONCLUSIONS

Results from the present study in broiler breeders show that reducing dietary CP level by 15 g/kg (on average from 140 to 125, depending on the breeder layer diet) reduces nitrogen excretion in the litter and manure by 8%, ammonia emission by 9%, total N losses by 11%, and does not affect water or feed intake. Overall, reducing CP level in the diet of broiler breeders reduces ammonia emission from litter and manure by 6% per 10 g/kg reduction of CP.

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