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Effects of diet density and feeding frequency during the rearing period on broiler breeder performance

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ABSTRACT

1. An experiment was conducted to study the effects of diet density and feeding frequency during the rearing period on broiler breeder performance between three and 40 weeks of age.
2. A total of 960 female one-day-old chicks (Ross 308) were randomly allocated to 24 floor pens (12 pens in two rooms). On day 21 pullets were assigned to a 2 × 2 factorial arrangement including two diets (control (CON) or 16% diluted (DIL)) and two feeding strategies (fed once (FO) or twice (FT) a day). The FO pullets were fed at 0815 h (100%) and FT pullets at 0815 h (60%) and 1215 h (40%). Water was provided by nipple drinkers with drip cups during 7 and 8 h in the rearing and laying period, respectively.
3. Body weight (BW) and water intake were measured weekly and BW uniformity at 10 and 20 weeks of age. Litter characteristics were measured at 10, 15, and 20 weeks of age. During the laying period, egg production and incubation characteristics were recorded.
4. The DIL pullets received a higher feed allowance in combination with similar water intake which resulted in a lower water/feed ratio compared to the CON pullets resulting in a higher DM content of the litter and improved litter quality. The higher feed allowance resulted in a 20% higher total manure production at 20 weeks of age. The FT pullets showed a lower body weight (BW) CV at 10 weeks of age; however, no effect was found at 20 weeks of age. During the laying period, FT pullets tended to have earlier onset of lay, higher total egg production at 30 weeks of age and better fertility.
5. It was concluded that alternative feeding strategies can positively influence production performance during both the rearing and laying period.

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Parent stock; nutrition; uniformity; litter quality; water/feed ratio; egg production; manure production

Introduction

For decades, broiler breeders have been selected for production of fertile eggs and for growth rate and efficiency in their offspring (de Jong and van Emous 2017). As a result, Zuidhof et al. (2014) showed that, over a 56-day growth period, a 2005 broiler strain grew 4.6 times the rate of a 1957 strain. Although a high growth rate and efficiency is desirable in broilers, it may lead to problems in parent stock during the rearing and laying period. *Ad libitum* feeding of breeder pullets, as in broilers, results in a two and half times higher BW with adverse effects on reproductive performance (Katanbaf et al. 1989; Savory et al. 1993; Heck et al. 2004). Heck et al. (2004) applied *ad libitum* feeding during the rearing period and found a five times higher mortality, 34% lower egg production and a 74% higher feed conversion ratio between 32 and 40 weeks of age, in comparison to restricted fed birds. To prevent these adverse effects, feed restriction during the pullet period is necessary (Mench 2002; D'Eath et al. 2009). Feed restriction usually means that breeder pullets receive a limited amount of feed, once a day in the morning, and birds display less than 1 h of effective feeding behaviour per day (de Jong and van Emous 2017). As a result, breeder pullets show behavioural signs of stress and frustration (especially during the rearing period) that are indicative of hunger, such as stereotypic object pecking and over-drinking (Savory and Kostal 1996; Hocking et al. 1996, 2001; de Jong et al. 2002). The discrepancy between aiming for a healthy and productive life and negatively affecting behaviour and welfare is referred to as 'the broiler breeder paradox' (Decuyper et al. 2010).

Several studies have tried to elucidate whether feeding diets with a low nutritional density or feeding multiple times per day could improve this paradox (e.g. de Jong et al. 2005a, 2005b; Nielsen et al. 2011; van Emous et al. 2014, 2015; Morrissey et al. 2014; de los Mozos et al. 2017). These studies, however, mostly focused on the effects of these interventions on behaviour and welfare. Only a few studies have been published investigating the effects of feeding strategies during the rearing period on the performance of broiler breeders during subsequent rearing and laying periods. Studies in which pullets were fed diluted diets showed that birds compensated for the lower nutrient concentration by consuming more feed to reach BW target at the end of the rearing period, without affecting BW uniformity (Enting et al. 2007b; Zuidhof et al. 2015; de los Mozos et al. 2017). Feeding low-density diets during the laying period increased laying and egg weight as compared to control diets in an experiment by Enting et al. (2007a). Feeding breeder pullets twice a day reduced BW uniformity at 13 weeks of age and increased feed intake as compared to feeding once a day in a study by van der Haar and van Voorst (2001).

To date, the influence of a combination of dietary treatments and feeding strategies on broiler breeder performance has not been studied. Therefore, the following work was conducted to determine the effects of feeding pullets two diets (control, diluted) at two frequencies (once a day or twice a day) in a 2 × 2 factorial arrangement on broiler breeder performance between 3 and 40 weeks of age.

Materials and methods

Experimental design

A total of 960 female one-day-old chicks (Ross 308) were randomly allocated to 24 floor pens (12 pens in two rooms). On day 21 pullets were assigned to a 2 × 2 factorial arrangement including two diets (control (CON) or 16% diluted (DIL)) and two feeding frequencies (once (FO) or twice (FT) a day) with six replicates per treatment. Up to two weeks of age, pullets were fed *ad libitum*, and restricted amounts of mash feed were fed daily from week two onwards. All pullets received the same starter-1 diet during the first three weeks. From three weeks onwards, pullets received standard control diets (CON), according to the recommendations of the

breeder company, or diluted diets (DIL) containing oat hulls (Table 1).

Pullets were fed a five-phase feeding programme: starter-1 (up to three weeks of age), starter-2 (three to eight weeks of age), grower (eight to 17 weeks of age), pre-breeder-1 (17–20 weeks of age) and pre-breeder-2 diets (20–23 weeks of age). For the DIL diet, the starter-2, grower, pre-breeder-1 and pre-breeder-2 were diluted by 10%, 20%, 20% and 10%, respectively. Furthermore, pullets were fed either once (FO) or twice (FT) a day. The FO pullets were fed at 0815 h (100% of the daily feed allowance). The FT pullets were fed at 0815 h (60% of the daily feed allowance) and 1215 h (40% of the daily feed allowance). From 23 weeks of age onwards, all breeders received the same standard breeder-1 diet

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

Item	Starter-1 (0–21 d)	Starter-2 (22–56 d)		Grower (57–119 d)		Pre-breeder-1 (120–140 d)		Pre-breeder-2 (141–161 d)	
		CON ¹	DIL	CON	DIL	CON	DIL	CON	DIL
Ingredient									
Maize	400.0	405.0	358.6	380.0	291.9	390.0	300.3	390.0	345.2
Wheat	217.3	254.0	224.9	200.0	153.6	233.0	179.4	233.0	206.2
Wheat middlings	30.5	30.5	27.0	150.0	115.2	100.0	77.0	100.0	88.5
Maize gluten feed	20.0	30.0	26.6	120.0	92.2	77.9	60.0	77.9	68.9
Sunflower meal	33.7	58.0	51.4	80.0	61.5	81.9	63.1	81.9	72.5
Soybean meal	232.0	159.0	140.8	21.8	16.7	53.3	41.0	53.3	47.2
Oat hulls	10.0	10.0	123.8	10.0	239.9	10.0	238.3	10.0	124.1
Soya oil	13.2	12.5	11.2	4.5	3.5	12.2	9.4	12.2	10.8
Chalk	17.3	17.6	15.6	18.7	14.4	26.7	20.8	26.7	23.7
Monocalcium phosphate	9.3	10.0	8.9	3.0	2.5	3.6	2.8	3.6	3.2
Salt	2.5	2.5	2.7	2.3	1.9	2.6	2.1	2.6	2.4
Sodium carbonate	2.8	3.1	1.9	2.4	1.5	2.5	1.5	2.5	2.0
Premix rear ²	4.0	4.0	3.5	1.0	0.8	-	-	-	-
Premix lay ³	-	-	-	3.0	2.3	4.0	3.1	4.0	3.5
Maxifit ⁴	4.0	2.0	1.8	-	-	-	-	-	-
L-Lysine	1.5	0.2	0.1	1.5	1.0	1.0	0.6	1.0	0.8
DL-Methionine	1.3	1.0	0.7	0.9	0.6	0.8	0.4	0.8	0.6
L-Threonine	0.6	0.6	0.5	0.9	0.6	0.5	0.2	0.5	0.4
Calculated content⁵									
AME _n (MJ/kg)	11.72	11.72	10.55	10.89	8.71	11.30	9.04	11.30	10.17
Crude ash	63.7	62.1	60.1	57.7	54.8	64.2	59.8	64.2	62.0
Crude protein	189.4	166.0	152.1	137.4	115.9	140.0	118.0	140.0	129.0
Crude fat	44.7	44.0	40.0	39.0	32.1	45.0	36.7	45.0	40.9
Crude fibre	38.8	44.5	73.2	61.1	115.2	56.2	111.1	56.2	83.6
NSP	172.9	178.8	244.5	235.8	355.9	210.8	335.7	210.8	273.3
Dig. Lys	9.0	6.4	5.8	4.8	3.8	4.9	3.9	4.9	4.4
Dig. Met+Cys	7.0	6.2	5.5	5.3	4.2	5.3	4.2	5.3	4.8
Dig. Thr	6.3	5.5	5.0	4.5	3.6	4.3	3.4	4.3	3.9
Dig. Trp	1.9	1.6	1.5	1.1	1.0	1.2	1.1	1.2	1.1
Na	1.9	2.0	1.8	2.0	1.6	2.0	1.6	2.0	1.8
K	8.6	7.6	7.2	7.3	6.5	7.0	6.3	7.0	6.6
Cl	2.4	2.2	2.3	2.5	2.0	2.5	2.0	2.5	2.3
DEB (mEq/kg)	235	220	198	203	180	195	174	195	185
Calcium	10.0	10.0	9.0	9.0	7.2	12.0	9.6	12.0	10.8
Total phosphorus	6.3	6.4	5.8	5.9	4.8	5.5	4.4	5.5	5.0
Available phosphorus	4.1	4.1	3.7	3.2	2.6	3.2	2.6	3.2	2.9
Analysed content									
DM	888	881	882	880	888	882	889	882	886
Crude ash	62	56	55	52	50	58	55	58	57
Crude protein	195	166	152	138	113	140	119	140	128
Crude fat	49	46	42	43	41	49	43	49	47
Crude fibre	44	49	71	64	110	55	107	55	77
Total phosphorus	6.4	6.3	5.6	5.5	4.6	5.0	4.2	5.0	4.7

¹Density diets: CON = control diet; DIL = diluted diet.

²Provided per kilogram of complete diet: retinol, 10,050 IU; thiamine, 3.0 mg; riboflavin, 12.1 mg; niacin, 48.2 mg; choline, 281.4 mg; pantothenic acid, 15.1 mg; pyridoxine, 4.0 mg; folic acid, 1.6 mg; cyanocobalamin, 0.03 mg; cholecalciferol, 2,513 IU; α-tocopherol, 40.2 mg; biotin, 0.2 mg; menadione, 3.0 mg; iron, 64.3 mg; copper, 5.0 mg; manganese, 30.2 mg; zinc, 30.2 mg; iodine, 1.5 mg; selenium, 0.4 mg.

³Provided per kilogram of complete diet: retinol, 10,050 IU; thiamine, 3.0 mg; riboflavin, 10.1 mg; niacin, 30.2 mg; choline, 301.6 mg; pantothenic acid, 16.4 mg; pyridoxine, 4.0 mg; folic acid, 2.0 mg; cyanocobalamin, 0.03 mg; cholecalciferol, 1,508 IU; α-tocopherol, 100.5 mg; biotin, 0.3 mg; menadione, 3.0 mg; iron, 100.5 mg; copper, 15.1 mg; manganese, 100.5 mg; zinc, 20.1 mg; iodine, 2.0 mg; selenium, 0.3 mg.

⁴Provided per kilogram of complete diet: retinol, 10,050 IU; thiamine, 0.8 mg; riboflavin, 1.6 mg; niacin, 8.0 mg; choline, 80.4 mg; pantothenic acid, 4.0 mg; pyridoxine, 0.8 mg; folic acid, 0.4 mg; cyanocobalamin, 0.006 mg; ascorbic acid, 30.2 mg; α-tocopherol, 10.1 mg; biotin, 0.002 mg; menadione, 0.8 mg; iron, 24.1 mg; manganese, 20.1 mg; iodine, 0.08 mg.

⁵CVB matrix values (CVB, 2011) were used for diet formulation.

(11.93 MJ/kg AMEn; 14.8% CP; 0.58% dig. Lys; 0.56% dig. M + C; 3.0% Ca; 0.32% aP).

Housing and management

The broiler breeder chicks (Aviagen-EPI, Roermond, The Netherlands) were housed in 24 floor pens (2.5 × 2.0 m; 5.0 m²) in two identical climate-controlled rooms. An extra pen with 30 pullets was available to replace dead and culled pullets between day 1 and 21. The study started at d 21 with 40 pullets/pen. Males were reared commercially and introduced into the pens (three males/pen) when the breeders were 20 weeks of age. The pens consisted of a litter floor area (4.17 m²) with wood shavings (2.0 kg/m²) and an elevated floor (0.83 m²) with plastic slats and three plastic perches (6 m total). Feed was provided manually on three feed trays up to week two, in three standing feeders between week two and six and in two feeding troughs for females (4.1 m length) from week six onwards. On week 20 a male exclusion system was installed onto the female feeders. A separate feeding trough (60 cm) was available for males, from 20 weeks of age onwards, positioned at a height of 50 cm, to prevent females from access to the feed. Water was supplied in seven nipple drinkers with drip cups above the litter floor until week six and above the slatted floor after that. Water was available between 0815 and 1530 h during the rearing period and between 0815 and 1630 h during the laying period. Outside each pen, adjacent to the slats, one nest box (88 × 36 cm) was placed at 22 weeks of age.

All birds in the different treatments were maintained at the same target body weight (BW). Feed allocation was adjusted to the predetermined body growth curve during the rearing period and a combination of the predetermined body growth and egg production curves during the laying period (Aviagen-EPI 2017). Males received a commercial male diet (10.89 MJ/kg AMEn; 13.0% CP; 0.45% dig. Lys; 0.5% dig. M + C; 1.0% Ca; 0.3% aP). Males and females were fed simultaneously.

Room temperature was maintained at 35°C during the first two days, and, from day three, was gradually reduced to 20°C at week four. The pullets were reared at a photoperiod of 24L:0D (40lx) for the first three days, which was gradually reduced to 8L:16D (5lx) at week three (lights on between 0745 and 1545 h). Breeders were photo stimulated with 10 h light at 21 weeks of age (40lx), and day length was gradually increased by 1 h (later 0.5 h) per week to 14L:10D at week 26 (lights on from 0245 to 1645 h). This photoperiod was maintained until the end of the study, at 40 weeks of age. Pullets were non beak trimmed and vaccinated according to a standard, commercial protocol supplied by Aviagen-EPI, Roermond, The Netherlands.

The protocol for the experiment agreed with standards for animal experiments and was approved by the Ethical Committee of Wageningen University and Research, Wageningen, The Netherlands (protocol number: AVD4010020185007).

Body weight and uniformity

To determine BW and weight gain, 10 females were randomly selected and weighed as a group, in the morning before feeding, weekly from one to 30 weeks of age and biweekly from 30 to 40 weeks of age. Individual BW of all hens was recorded at 10 and 20 weeks of age. BW uniformity

was expressed as coefficient of variation (CV) and calculated by dividing the standard deviation within the pen by the average body weight of each pen.

Water intake and water/feed ratio

Water intake was measured weekly per pen and cumulatively for the rearing and laying period were calculated. Weekly water/feed ratio was calculated for the rearing and laying period by dividing cumulative water intake by cumulative feed intake for each phase.

Litter characteristics

Litter quality was visually determined at 10, 15, and 20 weeks of age. Friability was scored on a 0 (% cake) to 5 (100% cake) point scale. Wetness of the litter in each pen was scored on a 0 (very dry) to 4 (very wet) point scale. The dry matter (DM) content of the litter was determined in duplicate litter samples per pen per point of time. Each litter sample was approximately 500 g and was taken from the full depth of the litter. Samples were taken from multiple locations within the pen to include spatial variation in the sample. The samples were mixed thoroughly, and 200 g was subsampled and weighed after oven drying for 4 h at 103°C (±3°C) (NEN 7432, 1996).

At 20 weeks of age, all litter (fresh faeces and wood shavings) and manure (under the slats) were removed from the pens and the mass of litter and manure was determined separately.

Egg production

Eggs were collected, graded and recorded per pen daily. The total number of settable (above 50 g), small (under 50 g), double yolked, abnormal shell, dirty and floor eggs were calculated per pen, per week and for the entire production period. Once a week, all hatching eggs (settable and small) were weighed. Average egg weight from the entire laying period was calculated. Age at sexual maturity (ASM) was defined as the age at which 50% of birds had become productive and was determined by linear interpolation within the week (in days) in which birds passed the level of 50% in lay. Peak egg production was determined as a three-week rolling average.

Incubation

Incubation characteristics were measured at 33 weeks of age. Due to unforeseen circumstances, only limited space was available in the incubator to measure these characteristics; therefore, 30 instead of 100 eggs per pen were collected from two days production, stored for six to seven days at 16°C to 18°C and 50% to 60% RH, transported, and set. At day 18 of incubation, eggs were transferred to an experimental room (for on-farm hatching) where they were further incubated at a temperature of 32°C to 33°C for the last three days of the incubation period. At day 21 of incubation, the number of hatched chicks were counted. Unhatched eggs were opened to distinguish eggs with embryonic mortality from unfertilised eggs. Fertility was assessed by the percentage of fertilised eggs (i.e. hatched eggs + unhatched eggs with embryonic mortality), calculated as:

$$\text{Fertility}\% = \frac{([\text{set eggs}] - [\text{unfertilised eggs}])}{[\text{set eggs}] \times 100}$$

Hatchability was determined for set eggs as a percentage by the calculation:

$$\text{Hatchability}\% = \left(\frac{[\text{hatched eggs}]}{[\text{fertile eggs}]} \right) \times 100$$

Embryonic mortality was calculated as:

$$\text{Embryonic mortality}\% = \left(\frac{[\text{unhatched eggs with embryonic mortality}]}{[\text{fertilized eggs}]} \right) \times 100$$

All chicks were scored as either first- or second-grade chicks. A first-grade chick was defined as dry, free of deformities and with bright eyes (Tona et al. 2004).

Statistical analysis

The data were analysed using Genstat (GenStat, 18th edition; Lawes Agricultural Trust, VSN International Ltd., Oxford, UK). Statistical significance difference was declared at $P \leq 0.05$, with $0.05 < P < 0.10$ considered as a tendency. Response variables with regard to litter (litter and manure production), egg production and incubation characteristics were analysed using the ANOVA (Analysis of Variance) procedure of GenStat according the following mixed model:

$$Y_{ijkl} = \mu + R_i + D_j + F_k + D_j \times F_k + \varepsilon_{ijkl}$$

where Y_{ijkl} was the response variable, μ the overall mean, R_i the random effect of room ($i = 1, 2$), D_j the fixed effect of diet density (CON, DIL; $j = 1, 2$), F_k the fixed effect of feeding frequency (FO, FT; $k = 1, 2$), $D_j \times F_k$ the interaction between diet density and feeding frequency, and ε_{ijkl} the residual error term.

The statistical model for BW uniformity, water intake, water/feed ratio and DM litter included age or phase as fixed effects, and pen was the experimental unit. Effects of diet density and feeding frequency on litter friability and wetness were analysed as separate Chi-Square Tests using the SPSS Statistics software (IBM 2018). Model parameters were tested for normal distribution before analysis. Apart from one response variable (litter production; Table 4), no statistically significant interactions were found between diet density and feeding frequency. Therefore, only main effects of treatments are reported and discussed.

Results

Body weight and uniformity

Results for BW and its uniformity in the pullets are presented in Table 2. DIL pullets had a 1.2% higher BW than CON pullets ($P < 0.001$) at 10 weeks of age. At 20 weeks of age however, DIL pullets had a 1.2% lower BW than CON pullets ($P = 0.048$). FT pullets had a 1.8% higher BW at 10 weeks of age ($P < 0.001$) and a 1.6% higher BW at 20 weeks of age ($P = 0.009$) than FO pullets. No significant interactions were found between diet density and feeding frequency.

No significant differences were found in BW uniformity between pullets fed the DIL or the CON diet; neither at 10 nor at 20 weeks of age. The FT pullets

showed a 2.7-% points lower CV value (i.e. higher BW uniformity) at 10 weeks of age ($P < 0.001$) compared to the FO pullets. At 20 weeks of age, the differences amounted 0.6-% points, but was not statistically significant.

Water intake and water/feed ratio

Results for water intake and water/feed ratio for both the rearing and laying period are presented in Table 3. To meet target body weight, DIL pullets received a 16% higher daily feed allowance than CON pullets between three and 23 weeks of age. Water intake of DIL pullets was not significantly different from CON pullets. As a result of the higher feed allowance at the same water intake, DIL pullets had a lower water/feed ratio ($P < 0.001$). No significant differences were found for water intake or water/feed-ratio between FT and FO pullets during the rearing period.

During the laying period, when hens from all four treatments in the rearing period (DIL, CON; FT, FO) received the same feed, feed allowance and feeding frequency (once a day), no carry-over effects were seen for water intake and water/feed ratio.

Litter and manure

Results of litter characteristics are presented in Table 4. Compared to CON pullets, the litter of DIL pullets had a higher DM content ($P < 0.001$) and tended to a lower friability score ($P = 0.061$) and a lower wetness score ($P = 0.080$). The DIL pullets produced 43% more litter ($P < 0.001$), but only a statistically undistinguishable amount was recovered under the slats (manure), resulting in a 20% higher total manure production ($P < 0.001$).

Table 2. The effects of diet density, feeding frequency and their interactions on BW (g) and coefficient of variation (CV (%)) at 10 and 20 weeks of age in broiler breeder pullets.

Source ¹	10 weeks of age		20 weeks of age	
	BW (g)	CV (%)	BW (g)	CV (%)
Diet density				
CON	1,198 ^b	12.2	2,350 ^a	10.5
DIL	1,212 ^a	13.2	2,323 ^b	10.5
SEM (n = 12)	2.3	0.50	9.2	0.32
Feeding frequency				
FO	1,194 ^b	14.2 ^a	2,318 ^b	10.8
FT	1,216 ^a	11.5 ^b	2,355 ^a	10.2
SEM (n = 12)	2.3	0.50	9.2	0.32
Treatments				
CON/FO	1,188	13.4	2,321	10.4
CON/FT	1,208	11.0	2,380	10.5
DIL/FO	1,200	15.0	2,315	11.1
DIL/FT	1,225	11.5	2,330	9.8
SEM (n = 6)	3.3	0.71	13.0	0.45
P-value				
Diet density	<0.001	0.158	0.048	0.994
Feeding frequency	<0.001	<0.001	0.009	0.190
Diet density × Feeding frequency	0.531	0.427	0.107	0.138

^{a,b}Means within a column with different superscripts differ statistically significant ($P \leq 0.05$).

¹CON = control diet; DIL = diluted diet; FO = fed once a day; FT = fed twice a day.

Table 3. The effects of diet density, feeding frequency and their interactions on water intake, feed intake, and water/feed ratio during the rearing and laying period in broiler breeder.

Source ¹	Rearing period (3–23 weeks of age)			Laying period (24–40 weeks of age)		
	Water intake (ml/bird/d)	Feed allowance (g/bird/d)	Water/feed ratio (ml/g)	Water intake (ml/bird/d)	Feed allowance (g/bird/d)	Water/feed ratio (ml/g)
Diet density						
CON	188.3	76.6	2.50 ^a	300.1	153.8	1.96
DIL	187.2	89.3	2.11 ^b	306.3	153.8	2.00
SEM (<i>n</i> = 12)	2.44	N.d. ²	0.03	2.83	N.d.	0.018
Feeding frequency						
FO	185.5	83.0	2.27	302.9	153.8	1.98
FT	190.0	83.0	2.33	303.5	153.8	1.98
SEM (<i>n</i> = 12)	2.44	N.d.	0.03	2.83	N.d.	0.018
Treatments						
CON/FO	186.1	76.6	2.47	301.0	153.8	1.97
CON/FT	190.5	76.6	2.53	299.2	153.8	1.95
DIL/FO	185.0	89.3	2.08	304.8	153.8	1.99
DIL/FT	189.5	89.3	2.14	307.8	153.8	2.01
SEM (<i>n</i> = 6)	3.46	N.d.	0.05	4.01	N.d.	0.026
<i>P</i> -value						
Diet density	0.762	N.d.	<0.001	0.135	N.d.	0.148
Feeding frequency	0.212	N.d.	0.221	0.879	N.d.	0.863
Diet density × Feeding frequency	0.996	N.d.	0.928	0.545	N.d.	0.530

^{a,b}Means within a column with different superscripts differ statistically significant ($P \leq 0.05$).

¹CON = control diet; DIL = diluted diet; FO = fed once a day; FT = fed twice a day.

²Not determined; differences in feed allowance between treatments were due to the design of the study.

Table 4. The effects of diet density, feeding frequency and their interactions on DM litter, friability and wetness, and litter, manure and total manure production in broiler breeder pullets¹.

Source ²	DM litter (g/kg)	Friability ³	Wetness ⁴	Litter (kg) ⁵	Manure (kg) ⁵	Total (kg)
Diet density						
CON	699.0 ^b	0.53	0.64	79.2 ^b	74.0	153.2 ^b
DIL	751.2 ^a	0.00	0.36	113.4 ^a	70.2	183.6 ^a
SEM (<i>n</i> = 12)	8.67	N.d. ⁶	N.d.	2.61	4.30	5.00
Feeding frequency						
FO	734.4	0.22	0.47	102.2 ^a	62.9 ^b	165.0
FT	715.7	0.31	0.53	90.4 ^b	81.3 ^a	171.7
SEM (<i>n</i> = 12)	8.67	N.d.	N.d.	2.61	4.30	5.00
Treatments						
CON/FO	710.8	N.d.	N.d.	89.8 ^b	63.1	152.8
CON/FT	687.1	N.d.	N.d.	68.7 ^c	84.8	153.5
DIL/FO	758.1	N.d.	N.d.	114.6 ^a	62.7	177.2
DIL/FT	744.3	N.d.	N.d.	112.2 ^a	77.7	189.9
SEM (<i>n</i> = 6)	12.26	N.d.	N.d.	3.69	6.07	7.07
<i>P</i> -value						
Diet density	<0.001	0.061	0.080	<0.001	0.544	<0.001
Feeding frequency	0.143	0.181	0.886	0.005	0.007	0.358
Diet density × Feeding frequency	0.687	N.d.	N.d.	0.020	0.590	0.407

^{a,b}Means within a column with different superscripts differ statistically significant ($P \leq 0.05$).

¹DM litter, friability and wetness represents the average of 10, 15 and 20 weeks of age. Litter, manure and total manure production is measured at 20 weeks of age.

²CON = control diet; DIL = diluted diet; FO = fed once a day; FT = fed twice a day.

³Friability was scored on a 0 (% cake) to 5 (100% cake) point scale.

⁴Wetness of the litter was scored on a 0 (very dry) to 4 (very wet) point scale.

⁵Litter = total fresh faeces and wood shavings and manure = fresh faeces under the slats.

⁶Not determined due to chi-square test.

No significant differences were found between FT and FO pullets for litter DM content, friability or wetness. The FT pullets produced less fresh faeces in the litter and more on the slats resulting in 12% less litter ($P = 0.005$) but 29% more manure ($P = 0.007$) than FO pullets. The net result of those differences, expressed as total manure production, was not statistically different between FT and FO pullets.

Egg production

Results for egg production are presented in Tables 5 and 6. No statistically significant differences were found between breeders fed the DIL and CON diets during the rearing period for any of the egg production characteristics (Table 5) between 23 and 40 weeks of age. Breeders fed

twice a day during the rearing period tended to have a 5% higher total number of eggs ($P = 0.077$) between 23 and 30 weeks of age as compared to the breeders fed once a day during the rearing period.

For egg weight and age at peak egg production, no significant differences were found between breeders fed the DIL and CON diets during the rearing period (Table 6). Breeders fed twice a day during the rearing period tended towards a 0.8 days advanced ASM as compared to breeders fed once a day during the rearing period ($P = 0.053$).

Incubation

Results for incubation characteristics and chick production are presented in Table 7. No significant differences were

Table 5. The effects of diet density, feeding frequency and their interactions on egg production characteristics (number/hen) in broiler breeders between 23 and 40 weeks of age.

Source ¹	Total eggs 23–30 weeks of age	Total eggs 23–40 weeks of age	Settable eggs ²	Double yolk eggs	Abnormal shell eggs ³	Dirty eggs	Floor eggs ⁴
Diet density							
CON	17.8	77.3	65.6	1.0	1.5	3.9	3.8
DIL	18.0	77.1	65.2	1.1	1.7	3.7	3.7
SEM (<i>n</i> = 12)	0.30	0.74	1.31	0.08	0.12	0.41	0.77
Feeding frequency							
FO	17.5	77.0	65.2	1.0	1.5	3.6	4.0
FT	18.3	77.4	65.6	1.1	1.6	4.0	3.5
SEM (<i>n</i> = 12)	0.30	0.74	1.31	0.08	0.12	0.41	0.77
Treatments							
CON/FO	17.6	77.1	65.9	1.0	1.5	3.4	3.7
CON/FT	18.0	77.5	65.3	1.1	1.4	4.4	3.9
DIL/FO	17.4	76.9	64.6	1.0	1.5	3.8	4.4
DIL/FT	18.6	77.3	65.8	1.1	1.8	3.7	3.1
SEM (<i>n</i> = 6)	0.43	1.05	1.85	0.11	0.17	0.57	1.10
<i>P</i> -value							
Diet density	0.678	0.817	0.842	0.789	0.260	0.788	0.952
Feeding frequency	0.077	0.680	0.870	0.617	0.490	0.453	0.616
Diet density × Feeding frequency	0.423	0.988	0.630	0.939	0.212	0.320	0.543

^{a,b}Means within a column with different superscripts differ statistically significant ($P \leq 0.05$).

¹CON = control diet; DIL = diluted diet; FO = fed once a day; FT = fed twice a day.

²Settable eggs = normal egg ≥ 50 g.

³Abnormal shell eggs = cracked and soft shell.

⁴Floor eggs = eggs laid outside nest boxes (in litter or on slats).

Table 6. The effects of diet density, feeding frequency and their interactions on egg weight, ASM, peak egg production, and age at peak egg production in broiler breeders.

Source ¹	Egg weight ² (g)	ASM ³ (d)	Peak egg production (%) ⁴	Age at peak egg production (d)
Diet density				
CON	59.8	193.5	88.4	237.7
DIL	59.8	193.2	88.1	235.1
SEM (<i>n</i> = 12)	0.17	0.29	0.80	2.6
Feeding frequency				
FO	59.7	193.8	87.9	238.6
FT	59.9	193.0	88.5	234.2
SEM (<i>n</i> = 12)	0.17	0.29	0.80	2.6
Treatments				
CON/FO	59.7	193.8	88.1	243.8
CON/FT	59.8	193.2	88.6	231.6
DIL/FO	59.7	193.8	87.7	233.3
DIL/FT	60.0	192.7	88.4	236.8
SEM (<i>n</i> = 6)	0.24	0.41	1.13	3.7
<i>P</i> -value				
Diet density	0.760	0.456	0.801	0.482
Feeding frequency	0.393	0.053	0.568	0.247
Diet density × Feeding frequency	0.895	0.525	0.927	0.145

¹CON = control diet; DIL = diluted diet; FO = fed once a day; FT = fed twice a day.

²Egg weight was determined for all hatching eggs (small and settable).

³ASM = age at sexual maturity, defined as age at 50% production.

⁴Determined as a 3-weeks rolling average of % hen day.

found between DIL and CON pullets, although the FT pullets tended to a 3.6-% points higher fertility compared to the FO pullets ($P = 0.080$).

Discussion

Effect of diet density

The BW of pullets in the rearing period differed between DIL and CON treatments by only 14 g, or 1.2%, at 10 weeks of age and by only 27 g, or 1.6%, at 20 weeks of age. Based on the number of observations and the relatively small SEMs, however, these differences proved statistically significantly. Since differences were small and inconsistent in time, it was

postulated that this might not represent a true effect, or, if it did, the relevance in terms of technical performance on pullets was small.

The BW uniformity was not affected by diet density at 10 and 20 weeks of age. This was in agreement with the previous results reported by Zuidhof et al. (2015) and de los Mozos et al. (2017) who used 23% and 15% diluted diets, respectively. Pullets fed the 16% diluted diets needed 16% more feed to compensate for the lower levels of nutrients in the diluted diet to obtain similar BW, which has been previously observed in studies of Enting et al. (2007b), Zuidhof et al. (2015), and de los Mozos et al. (2017).

By diluting the diets, it was expected that water intake would increase due to the higher feed allowance and the

Table 7. The effects of diet density, feeding frequency and their interactions on fertility, hatch of set eggs, hatch of fertile eggs, total embryonic mortality and second-grade chicks from 33 weeks of age broiler breeders.

Source ¹	Fertility (%)	Hatchability of set eggs (%)	Hatchability of fertile eggs (%)	Total embryonic mortality (%)	Second-grade chicks ² (%)
Diet density					
CON	95.4	85.4	89.8	10.2	5.2
DIL	96.7	89.2	92.5	7.5	2.1
SEM (<i>n</i> = 12)	1.36	2.46	2.08	2.08	1.37
Feeding frequency					
FO	94.3	86.2	91.3	8.7	2.8
FT	97.9	88.4	90.9	9.1	4.4
SEM (<i>n</i> = 12)	1.36	2.46	2.08	2.08	1.37
Treatments					
CON/FO	93.4	82.5	88.2	11.8	4.3
CON/FT	97.4	88.4	91.4	8.6	6.1
DIL/FO	95.1	89.9	94.4	5.6	1.4
DIL/FT	98.3	88.5	90.5	9.5	2.8
SEM (<i>n</i> = 6)	1.92	3.47	2.95	2.95	1.94
<i>P</i> -value					
Diet density	0.503	0.298	0.383	0.383	0.122
Feeding frequency	0.080	0.531	0.893	0.893	0.422
Diet density × Feeding frequency	0.853	0.314	0.248	0.248	0.922

^{a,b}Means within a column with different superscripts differ statistically significant ($P \leq 0.05$).

¹CON = control diet; DIL = diluted diet; FO = fed once a day; FT = fed twice a day.

²Expressed as a percentage of total number of hatched chicks.

extra water that would be necessary for metabolic processes. However, in the present study, water intake was similar for DIL pullets and CON pullets. It was speculated that water intake for breeder pullets might have been maximised by the physical capacity of the crop (Wehner and Harrold 1982) and was not affected by feed allowance at levels close to this capacity. Crop size was not measured in this study to substantiate this explanation.

The similar water intake and higher feed intake in this study was associated with a 16% lower water/feed ratio and a 7.5% higher litter DM content for the DIL pullets. The lower water/feed ratio has most likely caused a higher DM content of the faeces and subsequently in the litter, which was in agreement with similar findings in broiler studies (Bailey 1999; van Harn et al. 2019).

Moreover, the DIL diets were formulated with a coarse, insoluble NSP ingredient (oat hulls; 12–24% inclusion), evidently increasing crude fibre content. The results suggested that high fibre positively influenced litter quality in terms of dryness. This was confirmed by results published by Hocking (2006) and Nielsen et al. (2011), who used diluted diets with oat hulls and reported a lower water intake and drier litter. Furthermore, Savory et al. (1996) provided pullets diets containing oat hulls and reported lower water content of excreta. Providing broiler breeder pullets with diets diluted with oat hulls resulted in an extended mean retention time (MRT) in the caeca, and a shorter MRT in the jejunum and colon (Enting et al. 2007b). A study in laying hens found that addition of coarsely ground NSP sources to the diet increased MRT in the foregut (van Krimpen et al. 2011). Insoluble NSP accumulates in the gizzard and is retained longer than other nutrients, probably because it has to be ground to a critical particle size before entering the small intestine (Hetland et al. 2002, 2003). Enlarged reflux in the gizzard probably causes more water to be removed from the digesta. Thus, prolonged MRT in the gizzard and caeca probably resulted in a higher DM content of the excreta, which subsequently improved litter quality.

Despite the relatively high water/feed ratio, litter was dry and friable throughout the rearing period, with statistically significant drier and more friable litter for the DIL pullets. These differences were due to the higher DM content of the litter, caused by the higher feed intake at unchanged water intake. Better litter quality (drier and more friable) could have had beneficial side-effects on leg health, such as the incidence of foot pad dermatitis (Kaukonen et al. 2016). In line with expectations, the 16% higher feed intake and similar water intake resulted in more fresh faeces resulting in 20% higher litter and total manure production for DIL pullets.

One of the prominent welfare problems in breeder pullets caused by feed restriction is over-drinking, resulting in wet litter (Kostal et al. 1992; Savory et al. 1993; Hocking et al. 1996, 2001). The average water/feed ratio of 2.3 in the present study was higher than the value of 1.8 recommended under commercial conditions (Aviagen-EPI 2017). The results of the present study showed that high water intake did not necessarily result in wet litter, which suggested that the pullets did not over-consume water when feed restriction was applied. This is in contrast to results from other studies, which might have been due to factors unrelated to feed restriction, i.e. the type of water system and access to water. The first explanation could be that, in the present study, nipple drinkers with drip cups were used whereas, in previous studies, open water systems were used (Kostal et al. 1992; Savory et al. 1993; Hocking et al. 1996, 2001). For pullets, water intake from open water systems is much higher than from nipple drinkers, which causes over-drinking. Moreover, open water systems can lead to more water spillage, compared to nipple drinkers, leading to an incorrect assessment of the true water intake and may partly cause the problems with wet litter mentioned in previous studies. The second explanation is the longer access to the water system because of the longer day length (14 h) as used by Kostal et al. (1992) and Savory et al. (1993) compared to the present study (7 to 8 h), which can lead to compulsive drinking to prevent boredom.

Effect of feeding frequency

The BW of pullets in the rearing period in this study differed between FT and FO treatments by only 22 g, or 1.8%, at 10 weeks of age, and by only 37 g, or 1.6%, at 20 weeks of age. For feeding frequency, the higher BW of FT pullets was both significantly different and consistent in time. This pointed towards a causal effect of FT on BW but – as argued for diet density – the relevance of this finding in terms of technical performance of pullets was small.

In contrast with expectations, feeding pullets twice a day improved flock uniformity (11.5 vs. 14.2% BW CV) at 10 weeks of age (Table 2), albeit this difference diminished to a small and not significant level at 20 weeks of age. This finding contrasted with a study by van der Haar and van Voorst (2001), who found lower flock uniformity at 13 weeks of age when pullets were fed twice a day. This discrepancy between the present and aforementioned work may be explained by the differences in feed structure (mash and pellets) and/or the difference in breeds (Ross 308 and Ross 508) that were used. It was hypothesised that the improved BW CV at 10 weeks of age of the FT pullets was caused by physical factors. When feed is provided once a day, especially at the start of the rearing period between two and five weeks of age, heavier pullets typically have a physical advantage (due to a bigger crop) over lighter birds (Wehner and Harrold 1982), resulting in higher feed consumption. When feed is provided twice a day, the smaller pullets have the option to feed continuously before feed runs out. The finding that the difference in BW uniformity had diminished to a small and not significant level at 20 weeks of age may have been due to all pullets at that age having developed crop sizes sufficiently large enough to compete with other birds at the given level of feed allowance.

The FT pullets produced 29% more manure on the raised slatted floor than FO pullets. Although not presented in this paper, data from behavioural observations showed that FO pullets spent 27% more time on the slats performing roosting behaviour. It is known that broiler breeders have a fairly constant excretion of manure throughout the day (Farmer et al. 1983). Most likely, the extra time spent on the slats resulted in the greater accumulation of manure.

Feeding pullets twice a day resulted in a statistical tendency to both an advanced ASM and, probably as a result of this, 5% higher total egg production at 30 weeks of age. This higher egg production dropped to a lower and statistically insignificant difference at 40 weeks of age. Furthermore, feeding twice a day resulted in a 3.6% points higher fertility. Although body composition was not measured in the present study, feeding twice a day during the rearing period appeared to distribute nutrients in a more balanced manner throughout the day, as described by Backhouse and Gous (2006). This, in turn, may result in altered body composition (more fat and less protein) at the end of the rearing period and change technical performance (van Emous et al. 2015).

Conclusions

Diluting diets during the rearing period decreased the water/feed ratio, improved litter quality, but did not affect BW

uniformity. No carry-over effects were found on reproduction performance. Twice a day feeding during the rearing period improved BW uniformity. A carry-over effect was found for improved fertility and egg production during the early laying period. From these results, it was concluded that alternative feeding strategies can positively influence production performance during both the rearing and laying period of pullets.

Disclosure statement

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