Effects of twice a day feeding and split feeding during lay on broiler breeder production performance, eggshell quality, incubation traits, and behavior

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ABSTRACT Eggshell quality of broiler breeders' eggs decreases at the end of the laying period. Feeding a limited daily allowance of feed in the morning does not supply the necessary nutrients, particularly calcium for eggshell formation, at the right time of the day. Therefore, an experiment was conducted to study the effects of providing a standard diet twice a day or split feeding (special morning and afternoon diet) in broiler breeders on production performance, eggshell quality, incubation traits, and behavior. At 50 wk of age (**WOA**) 576 females and 48 males were randomly allotted to 24 floor pens and assigned to one of three treatments: 1) Standard breeder diet fed once a day (100% at 0730 h) (**CON1x**), 2) Standard breeder diet fed twice a day (50% at 0730 h and 50% at 1400 h) (**CON2x**), and 3) Split feeding fed twice a day, with a special morning (0730 h) and afternoon (1400 h) diet composition (**SP2x**). The morning diet was energetically comparable with the control diet, but it contained more protein and phosphorus (P) and less calcium (Ca). The afternoon diet had a lower energy, protein and P and a higher Ca content than the control and morning diet. The SP2x birds tended to have a higher egg production between 51 and 55 WOA (27.0 vs. 25.9 eggs; $P = 0.088$) compared to the CON1x birds, while the CON2x birds (26.6 eggs) did not differ from the other treatments. No differences were found on egg production for the total period (51−60 WOA) and on other production parameters. The different feeding strategies did not affect eggshell quality and incubation traits. However, the feeding strategies affected the behavioral patterns considerably. Twice a day feeding (CON2x and SP2x) resulted in more time spent on eating and sitting, and in less time spent on foraging and object pecking ($P \leq 0.05$) compared to feeding once a day (CON1x). In conclusion, twice a day feeding improves behavior and split feeding improves both egg production and behavior in broiler breeders, however, no effects were observed on eggshell quality and incubation traits.

Key words: broiler breeders, twice a day feeding, split feeding, eggshell quality, behavior

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INTRODUCTION

Despite an increased dietary calcium level while aging and/or additional calcium source provision (oyster shell or large limestone) in the afternoon, eggshell quality decreases with age in most broiler breeder flocks (Leeson and Summers, 2005). This is caused by the limited amount of daily feed fed to broiler breeders in the morning, which is usually consumed within 2 to 6 h (Roland and Farmer, 1984; Backhouse and Gous, 2005). This feeding practice, however, does not optimally support the breeder females nutrient requirements (e.g., Cave, 1981; Backhouse and Gous, 2006). The majority of eggs are laid in the morning (Zakaria et al., 2005; Zakaria and Omar, 2013) and the next ovulation occurs within 1 h (Etches, 1987). Egg production starts with approx. 6 h of albumen formation which requires mostly protein and amino acids (Leeson and Summers, 2005). Thereafter, eggshell formation takes places for approx. 18 h, which requires mostly calcium (e.g., Bootwalla et al., 1983). The morning feed is digested within approx. Four to 5 h before eggshell formation starts (Bar, 2008). Thus, during the eggshell formation, the necessary nutrients are not available from the feed, and must be subtracted from the bone (Bar, 2008). Dividing the single amount of standard feed into 2 portions during the day will improve the availability of nutrients in relation to egg formation and eggshell deposition (Farmer et al., 1983a), resulting in improved Ca utilization (Farmer et al., 1983b; Roland and Farmer, 1984). Previous studies on twice a day feeding

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resulted in higher eggshell weight (Lewis and Perry, 1988), however, no effects on eggshell weight and thickness were observed by Samara et al. (1996), Backhouse and Gous (2005), and Spradley et al. (2008). Besides the effect on eggshell quality different studies demonstrated that broiler breeder hens fed twice a day produce more eggs compared to breeders fed once a day (Spradley et al., 2008; Taherkhani et al., 2010; Moradi et al., 2013; Soltanmoradi et al., 2013), however, other authors did not observe such an effect (de Avila et al., 2003; Backhouse and Gous, 2005; Londero et al., 2015, 2016).

To improve the eggshell quality during the second half of the laying period, layer hens are fed specially formulated morning and afternoon diets which matches the different nutritional requirements during egg formation (de Los Mozos and Sanchez, 2014; Molnár et al., 2018). Birds fed via this split feeding program receive a diet that is tailored to meet the requirements for albumen formation in the morning (higher energy, protein and phosphorus, and lower calcium), and a different diet to facilitate eggshell formation in the afternoon (more calcium and less energy, protein and phosphorus). de Los Mozos and Sanchez, 2014 investigated split feeding in old laying hens (between 95 and 98 WOA) and observed a higher eggshell weight, thicker eggshell and 30% less cracked and shell less eggs. Research by van Krimpen et al. (2018) showed that split feeding in organic laying hens resulted in a lower phosphorus excretion without negative effects on egg production and eggshell quality. On the other hand, no effects of split feeding with layers in aviary systems were observed by Molnár et al. (2018). Till now, split feeding for broiler breeders has not been studied yet. Moreover, no studies have even been conducted to test the effect of twice a day feeding on the behavior of adult female breeders. Therefore, an experiment was performed to determine the effects of providing a standard diet twice a day or split feeding in broiler breeders on production performance, eggshell quality, incubation traits, and behavior.

MATERIALS AND METHODS

Experimental Design

In this experiment three different treatments (feeding strategies) were applied, each with 8 replicates: 1) birds fed the control diet (**CON1x**) (= standard breeder diet) once a day (0730 h), 2) birds fed the control diet (= standard breeder diet) twice a day (50% at 0730 h and 50% at 1400 h) (**CON2x**), and 3) birds fed according to split feeding, 2 different diets (a morning diet at 0730 h and an afternoon diet at 1400 h) (**SP2x**) (Table 1). The morning diet was energetically comparable with the control diet, but it contained more crude protein and phosphorus (**P**) and less calcium (**Ca**). The afternoon diet had a lower energy, crude protein and P content and a higher Ca content compared to the control and morning diet.

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

Item	Control diet	Split feeding morning diet	Split feeding afternoon diet
Ingredient			
Maize	358.2	358.2	358.2
Wheat	314.3	310.7	302.1
Rapeseed expeller	55.1	29.6	29.6
Rapeseed meal	24.5	50.0	50.0
Sunflower meal	110.0	119.7	99.2
Soybean meal	10.0	19.9	10.0
Palm oil	10.0	10.0	10.0
Soya oil	10.2	9.3	8.0
Salm oil	4.0	4.0	4.0
Salcurb Dry K2	7.5	7.5	7.5
Limestone	71.5	57.7	99.5
Chalk	10.0	10.0	10.0
Monocalcium phosphate	1.7	1.4	-
Salt	1.4	1.6	1.5
Sodium carbonate	2.7	2.5	2.4
Premix lay[1]	4.0	4.0	4.0
DL-Methionine	0.5	0.5	-
L-Lysine	1.1	0.5	0.8
L-Threonine	0.8	0.5	0.6
m2342 GLU-XYL	2.5	2.5	2.5
Calculated content[2]			
AMEn (kcal/kg)	2,760	2,766	2,669
Crude ash	110.8	98.0	136.1
Crude protein	133.5	140.0	127.5
Crude fat	49.1	47.0	45.0
Crude fiber	45.0	47.0	43.0
Starch	430.6	430.2	423.8
Dig. Lys	4.90	4.83	4.47
Dig. Met+Cys	5.18	5.43	4.48
Dig. Thr	4.66	4.58	4.25
Dig. Trp	1.27	1.37	1.21
C18:2 Linolenic acid	16.50	15.90	14.90
Sodium	1.40	1.40	1.40
Potassium	5.49	5.85	5.32
Chloride	1.60	1.60	1.60
dEB (mEq/kg)	156.0	166.0	152.0
Calcium	34.0	28.7	44.5
Total phosphorus	4.16	4.24	3.61
Available phosphorus	2.80	3.03	2.18
Analyzed content			
DM	890.0	887.0	894.0
Crude ash	108.0	87.0	142.0
Crude protein	138.0	148.0	129.0
Crude fat	47.0	48.0	44.0
Crude fiber	43.0	51.0	39.0
Total calcium	37.4	27.2	53.2
Total phosphorus	4.43	4.92	3.96

[1]Provided per kilogram of complete diet: vitamin A, 10,050 IU; vitamin B1, 3.0 mg; vitamin B2, 12.1 mg; vitamin B3, 48.2 mg; vitamin B4, 281.4 mg; vitamin B5, 15.1 mg; vitamin B6, 4.0 mg; vitamin B9/B11, 1.6 mg; vitamin B12, 0.03 mg; vitamin D3, 2,513 IU; vitamin E, 40.2 mg; vitamin H, 0.2 mg; vitamin K3, 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.

[2]CVB matrix values (CVB, 2016) were used for diet formulation.

Housing and Management

The experiment was conducted between 50 and 60 WOA with Ross 308 broiler breeders available from a previous experiment. Before the start of the experiment, the females present were randomized and graded; birds that were underweighted, overweighed, not laying and injured were removed. In total, 576 females and 48 males were randomly allotted to 24 floor pens (2.5 × 2.0 m) in 2 identical climate-controlled rooms (12 pens per room). Both rooms included 4 complete replicates of each treatment.

Individual pens contained 24 females and 2 males. The pens contained an elevated floor (100 × 150 cm; 30% of the floor surface) with wooden slats and fresh wood shavings (2.0 kg/m^2) were used as litter on the remaining area. Female feed was provided manually in 2 feeding troughs (total 3.7 m length) with a male exclusion system. A separate feeding trough (60 cm) was available for the males positioned at a minimum height of 50 cm, to prevent female access to the feed. Water was supplied between 0730 and 1630 h by 7 nipple drinkers with drip cups above the slatted floor. Outside each pen, adjacent to the slats, one nest box (88 × 36 cm) was placed. During the experiment, all birds of the different treatments were maintained on the same target body weight (**BW**). Feed allocation was adjusted to the predetermined body growth curve and egg production (Aviagen-EPI, 2017). Males were fed once a day (0730 h) a commercial male diet (2,600 kcal/kg AMEn; 13.0% CP; 0.45% dig. Lys; 0.5% dig. M+C; 1.0% Ca; 0.3% aP). Additional oyster shell (500 g) was provided weekly in the feeding troughs. Room temperature was maintained at 20°C and the photoperiod was 14L:10D (40 lx), with lights on from 0245 to 1645 h. This study was approved by the Dutch Central Authority for Scientific Procedures on Animals (CCD) and is registered under application number AVD4010020185007.

Observations

Diet analysis: The experimental diets were formulated and produced by ABZ Diervoeding, Leusden, the Netherlands. Diets were analyzed for dry matter, crude ash (ISO 5984), crude protein (ISO 5983), crude fat (ISO 6492), crude fiber (ISO 6865), calcium (ISO 6869), and phosphorous (ISO6941). All analyses were done in duplicate and carried out by NutriControl, Veghel, the Netherlands.

Body weight: To monitor BW and BW gain, 10 females (as group) and males (individual) were weighed biweekly in the morning before feeding.

Production performance: All eggs per pen were collected daily, graded, and recorded. The total number of settable (above 50 g), small (under 50 g), double yolk, abnormal eggshell, dirty, and floor eggs were calculated per week and for the total experimental period on pen basis. On a weekly basis, on the same day of the week, all hatching eggs (settable and small) were weighed. Average egg weight of the experimental period was calculated.

Eggshell Quality

Eggshell thickness: At 56 and 59 WOA, eggshell thickness of 10 first grade eggs per pen was measured using a Mitutoyo 395-541(Mitutoyo Corporation, Japan). Eggshell thickness was determined at 3 locations of the egg: the top, the middle and the bottom and average eggshell thickness was calculated (according to van Krimpen et al., 2018).

Breaking strength and stiffness eggshell: At 56 and 59 WOA, maximum breaking strength and stiffness of the eggshell (a measure of flexibility) of 10 first grade eggs per pen were determined by using an Instron 5564 Texture Analyzer (Norwood, MA). The device determined the compression pressure at break (Newton), energy to break (J) and egg stiffness (N/mm).

Egg mottling: At 56 and 59 WOA, 30 first grade eggs per pen were scored using a flashlight for egg mottling between 1 (few and small) to 4 (many and large) (according to Vasileva et al., 2018). Egg mottling is a measure of the presence of translucent spots on the eggshell.

Albumen/yolk ratio and eggshell weight: At 51, 53, and 56 WOA, albumen/yolk ratio and eggshell weight of 10 first grade eggs per pen were determined. The eggs were first weighed fresh and then boiled for 10 min and weighed again, thereafter yolk and albumen were separated and weighed. Eggshell weight was determined immediately after boiling and after drying at room temperature for 24 h. Dry matter of the eggshell was calculated.

Incubation traits: Incubation traits were measured at 55 and 60 WOA. Per pen, 50 eggs (collected from 3 d of production) were, after a 5 to 7 d storage period (16−18°C and 50−60% RH), placed in an incubator. At d 7 of incubation, all eggs were opened to determine unfertilized eggs and age of embryonic mortality.

Behavior: Home pen behavior of the birds was observed by live scan sampling of each pen at 54 and 59 WOA. Behavior observations were performed by 2 pre-trained persons during the observation day consisting out of 8 observation sessions throughout the light period, focusing on the 2 feeding times. The first observation session started at 0800 h and was 30 min after feeding and sessions were repeated each hour until the last one at 1500 h. Before each observation session, 5 min of habituation time per compartment was apprehended and observers switched rooms between observation sessions. Behavior was scored by counting the birds performing different behaviors according to the ethogram previously described by van Emous et al. (2015) (Table 2). Eating and drinking was only recorded when feed and water was available. During the availability of feed, object pecking was defined as pecking at the pen or equipment and when feed troughs were empty pecking at the feeder was also scored as object pecking.

Statistical Analysis

The data were analyzed using Genstat statistical software (Genstat, 2018). Response variables with regard to production performance were analyzed using the ANOVA (Analysis of Variance) procedure according

Table 2. Ethogram of the behavioral observations (based on van Emous et al., 2015).

Behavior	Definition
Eating	Pecking at feed at the feeding troughs
Drinking	Pecking at water at the nipple drinkers
Standing	Standing without performing other behavior
Sitting	Sitting without performing other behavior
Walking	Walking or running without performing other behavior
Foraging	Pecking and/or scratching the litter
Comfort	All comfort behavior like, preening, auto pecking, nibbling, stroking, wing flapping and stretching
Dustbathing	Dustbathing behavior
Object pecking	Stereotypic pecking at parts of the pen, wall, empty feeding troughs, or empty nipple drinkers
Bird pecking	All pecking at other birds

the following model: $Y_{ijk} = \mu + R_i + FS_j + OS_k + \varepsilon_{ij}$, where Y_{ijk} is the response variable, μ the overall mean, R_i the random effect of room (i = 1, 2), FS_j the effect of feeding strategy (CON1x, CON2x, SP2x; j = 1..3), OS_k the effect of observation session (k = 1..8), and ε_{ij} the residual error term. The statistical model for incubation traits, eggshell quality and behavior included age as a fixed effect. Parameters were tested for normal distribution before analysis. After inspection of diagnostic plots of residuals, it was decided to analyze the behavioral variables with a logistic regression model. Pen was treated as the experimental unit. Statistical significance difference was declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered as a tendency.

RESULTS

Diet Composition

The analyzed crude protein content of the control, morning, and afternoon diets was respectively 3.4, 5.7, and 1.2% higher than the calculated content (Table 1). The analyzed crude fat content was lower for the control (−4.3%) and afternoon diet (−2.2%) and higher for the morning diet (+ 2.1%) than the calculated content. The analyzed phosphorus content was considerably higher for all feeds (on average 10.7%) than the calculated content. Even though the diets differed between the calculated and analyzed compositions, the desired contrast between the diets remained. The analyzed calcium content showed somewhat larger deviations compared to the calculated content (+10.0, −5.2 and + 19.6% respectively for the control, morning and afternoon diet). The desired contrast between the diets was therefore larger than expected, with lower calcium content in the morning diet and a higher content in the afternoon diet.

Body Weight

Body weight of the females did not differ between the treatments (data not shown). The males kept with the SP2x females were, on average, 500 g lighter ($P \leq 0.05$) throughout the experiment compared to the males kept with the CON2x females. The males that were kept with the CON1x females did not differ from the other treatments.

Production Performance

The SP2x birds tended to a higher egg production between 51 and 55 WOA compared to the CON1x (27.0 vs. 25.9 eggs; $P = 0.088$; Table 3) birds, while the CON2x birds (26.6 eggs) did not differ from the other treatments. There was no difference in egg production over the entire experimental period. No differences were found for other production characteristics and egg weight.

Eggshell Quality

No treatment effects on eggshell quality were observed, however, age had some effect on eggshell quality (Table 4). Hatching eggs at 59 WOA had a slightly thinner eggshell (0.330 vs. 0.336 mm; $P = 0.004$) than at 56 WOA. Furthermore, the eggs of breeders at 59 WOA tended to a higher level of egg mottling than the eggs of breeders at 55 WOA (1.58 vs. 1.48; $P = 0.077$).

No effects of treatments were observed on eggshell weight after cooking, eggshell weight after 24 h drying, eggshell DM content and albumen/yolk ratio (Table 5). There was an age effect with a lower albumen/yolk ratio at 56 WOA compared to 51 and 53 WOA (1.73 vs. 1.78; $P = 0.013$).

IncubationTraits

The treatments did not affect the different incubation traits (Table 6). Embryonic mortality on 3 to 4 d was lower for the 55 WOA compared to the 60 WOA hatching eggs (1.1 vs. 2.6%; $P = 0.021$), resulting in a lower total embryonic mortality at 55 WOA hatching eggs (1.3 vs. 3.0%; $P = 0.019$).

Behavior

The birds fed twice a day (average of CON2x and SP2x) spent more time on eating than the CON1x birds (33.6 and 33.8 vs. 31.0% for CON2x, SP2x and CON1x, respectively; $P = 0.040$; Table 7). Furthermore, there was a tendency for more time spent on sitting in SP2x compared to the CON1x (9.1% vs. 5.7%; $P = 0.069$), while the CON2x birds (8.1%) did not differ from the other treatments. In contrast, the birds fed twice a day spent less time on foraging (11.2 and 10.1% vs. 16.8% for CON2x, SP2x and CON1x, respectively; $P < 0.001$) and object pecking behavior (0.4 and 0.5% vs. 1.4% for CON2x, SP2x and CON1x, respectively; $P = 0.003$) than birds fed once a day. No treatment effects were observed for time spent on drinking, standing, walking, comfort, dustbathing, and bird pecking.

Table 3. The effects of the different feeding strategies on production performance.

Feeding strategy [1]	Total eggs (51−55 WOA) (#)	Total eggs (56−60 WOA) (#)	Total eggs (51−60 WOA) (#)	Hatching eggs (#)	Abnormal shell eggs (%)	Dirty eggs (%)	Floor eggs (%)	Egg weight (g)
CON1x	25.9	24.5	50.4	43.5	1.4	4.5	0.9	66.9
CON2x	26.6	25.4	52.0	45.6	1.1	3.2	1.9	67.0
SP2x	27.0	25.6	52.6	45.3	1.1	4.1	1.9	66.6
SEM (n = 8)	0.3	0.5	0.8	1.0	0.2	0.5	0.5	0.3
P-value	0.088	0.26	0.13	0.31	0.52	0.26	0.24	0.65

[1]CON1x, control diet once a day; CON2x, control diet twice a day; SP2x, split feeding.

Table 4. The effects of the different feeding strategies and age on eggshell thickness, breaking strength, stiffness, and egg mottling of hatching eggs.

Item[1]	Eggshell thickness (mm)	Breaking strength (Newton)	Breaking strength (Joule)	Stiffness (N/mm)	Egg mottling
Feeding strategy					
CON1x	0.337	39.0	0.0156	1.50	1.53
CON2x	0.331	37.8	0.0155	1.50	1.53
SP2x	0.331	37.8	0.0156	1.49	1.53
SEM (n = 8)	0.003	0.7	0.0006	0.03	0.05
Age					
56 WOA	0.336[a]	38.3	0.0153	1.48	1.48
59 WOA	0.330[b]	38.1	0.0159	1.51	1.58
SEM (n = 12)	0.001	0.5	0.0004	0.02	0.04
P-value					
Feeding	0.19	0.39	0.99	0.93	0.99
Age	0.004	0.75	0.32	0.42	0.077
Feeding × Age	0.086	0.58	0.33	0.67	0.55

[a-b]Means within a column with no common superscript differ ($P \leq 0.05$).
[1]CON1x, control diet once a day; CON2x, control diet twice a day; SP2x, split feeding.

Table 5. The effects of the different feeding strategies and age on eggshell weight, DM eggshell, and albumen/yolk ratio.

Item[1]	Eggshell weight after cooking (g)	Eggshell weight after 24 h drying (g)	DM eggshell (%)	Albumen/yolk ratio
Feeding strategy				
CON1x	6.81	6.08	89.2	1.77
CON2x	6.78	6.07	89.5	1.78
SP2x	6.70	6.01	89.3	1.74
SEM (n = 8)	0.05	0.04	0.3	0.02
Age				
51 WOA	6.75	6.03	89.0	1.78[a]
53 WOA	6.70	6.01	89.7	1.78[a]
56 WOA	6.84	6.12	89.3	1.73[b]
SEM (n = 8)	0.05	0.04	0.4	0.01
P-value				
Feeding	0.25	0.45	0.83	0.17
Age	0.11	0.14	0.43	0.013
Feeding × Age	0.70	0.53	0.71	0.91

[a-b]Means within a column with no common superscript differ ($P \leq 0.05$).
[1]CON1x, control diet once a day; CON2x, control diet twice a day; SP2x, split-feeding.

In addition, several age-related differences were found for behavior. The birds spent less time on eating and drinking and more time on standing, walking, foraging, comfort, and bird pecking at 59 vs. 54 WOA.

Considering the behavior during the observation days, the CON2x and SP2x treatments did not differ and therefore are combined as twice a day feeding against the once a day feeding (CON1x). Significant interactions ($P \leq 0.05$) between feeding frequency and observation sessions were found for all types of behavior (Figure 1). As expected, twice a day fed birds showed 2 peaks (morning and afternoon) for time spent on eating and drinking. The twice a day fed birds showed increased time spent on standing, which peaked at the sixth observation session (approx. 45% of the birds) and declined rapidly after the second feeding time. The once a day fed birds showed a slower, more linear increase in time spent on standing, plateauing at approx. 20% between the sixth and eighth observation session. Twice a day fed birds showed more time spent on sitting, with the peak

Table 6. The effects of the different feeding strategies and age on fertility and embryonic mortality (EM) at different stages.

Item[1]	Fertility (%)	EM 1−2 d (%)	EM 3−4 d (%)	EM 5−7 d (%)	Total EM (%)
Feeding strategy					
CON1x	94.9	0.1	1.9	0.3	2.3
CON2x	95.5	0.0	1.9	0.0	1.9
SP2x	97.6	0.7	1.8	0.0	2.4
SEM (n = 8)	1.1	0.2	0.5	0.1	0.5
Age					
55 WOA	95.9	0.2	1.1[b]	0.1	1.3[b]
60 WOA	96.1	0.4	2.6[a]	0.1	3.0[a]
SEM (n = 12)	0.9	0.1	0.4	0.1	0.3
P-value					
Feeding	0.16	0.12	0.98	0.11	0.78
Age	0.85	0.31	0.021	0.93	0.019
Feeding × Age	0.31	0.76	0.85	0.99	0.92

[a-b]Means within a column with no common superscript differ ($P \leq 0.05$).
[1]CON1x, control diet once a day; CON2x, control diet twice a day; SP2x, split feeding.

Table 7. The effects of the different feeding strategies and age on behavior (% of time).

Item[1]	Eating	Drinking	Standing	Sitting	Walking	Foraging	Comfort	Dust- bathing	Object pecking	Bird pecking
Feeding strategy										
CON1x	31.0[b]	14.0	13.3	5.7	8.6	16.8[a]	6.9	1.3	1.4[a]	1.0
CON2x	33.6[a]	12.3	17.1	8.1	7.9	11.2[b]	6.8	2.0	0.4[b]	0.6
SP2x	33.8[a]	13.0	16.6	9.1	7.9	10.1[b]	7.0	1.2	0.5[b]	0.9
SEM (n = 8)	0.8	0.7	1.4	1.0	0.5	0.7	0.7	0.3	0.2	0.2
Age										
54 WOA	34.6[a]	15.6[a]	14.6[b]	7.2	7.2[b]	11.6[b]	6.2[b]	1.5	0.9	0.5[b]
59 WOA	31.0[b]	10.6[b]	16.7[a]	8.1	9.1[a]	13.8[a]	7.6[a]	1.5	0.6	1.1[a]
SEM (n = 12)	0.5	0.4	0.7	0.6	0.4	0.5	0.4	0.3	0.2	0.1
P-value										
Feeding	0.040	0.26	0.15	0.069	0.50	<0.001	0.99	0.28	0.003	0.35
Age	0.001	<0.001	0.040	0.26	<0.001	0.003	0.028	0.93	0.16	0.002
Feeding × Age	0.65	0.34	0.85	0.53	0.44	0.60	0.72	0.72	0.16	0.36

[a-b]Means within a column with no common superscript differ ($P \leq 0.05$).
[1]CON1x, control diet once a day; CON2x, control diet twice a day; SP2x, split feeding.

of sitting (approx. 25%) around the fifth observation session. The once a day fed birds showed increasing time spent on sitting up to the fifth observation session, after which they stabilized between 5 and 10%. Walking behavior was barely influenced by feeding frequency and showed a comparable pattern for once and twice a day fed birds. Time spent on foraging behavior of the twice a day fed birds was higher at the second and third observation session, whereas it lowered from the fifth observation session onwards compared to the once a day fed birds. Time spent on comfort behavior increased more rapidly for the twice a day fed birds and was constant between the third and sixth observation session. After the second feeding at 1400 h, time spent on comfort behavior for the twice a day fed birds decreased to almost 0% while that for the once a day fed birds remained constant at 10%. With an exception at the third observation session, no treatment differences were observed in dustbathing behavior. During the first 6 observation sessions, no differences between the feeding strategies were found in object pecking. However, during the last 2 observation sessions, the twice a day fed birds spent less time on object pecking than the once a day fed birds. Despite more time was spent on bird pecking at the fourth and seventh observation session for the birds fed once a day, no clear pattern was observed.

DISCUSSION

Effect of Feeding Strategy on Production Performance

In the present study, despite a 1.6 numerical higher number of eggs, no significant effect of providing twice a day a standard diet was observed on egg production, which is in agreement with previous research (Cave, 1981; Lewis and Perry, 1988; Samara et al., 1996; de Avila et al., 2003; Backhouse and Gous, 2005; Londero et al., 2015, 2016). However, this is in contrast with the studies of Spradley et al. (2008), Taherkhani et al. (2010), Moradi et al. (2013), and Soltanmoradi et al. (2013), who observed an increased egg production when applying twice a day feeding. In general, the older studies (except Londero et al., 2015, 2016) showed no results on egg production whereas the more recent studies (from 2008 onward) showed a higher egg performance when breeders were fed twice a day. The inconsistency between the studies is probably caused by genetic differences. Modern broiler breeders have a higher growth potential than breeders 4 decades ago, resulting in increased feed restriction levels and longer fasting periods (de Jong and van Emous, 2017). Morris and Nalbandov (1961) showed that a prolonged

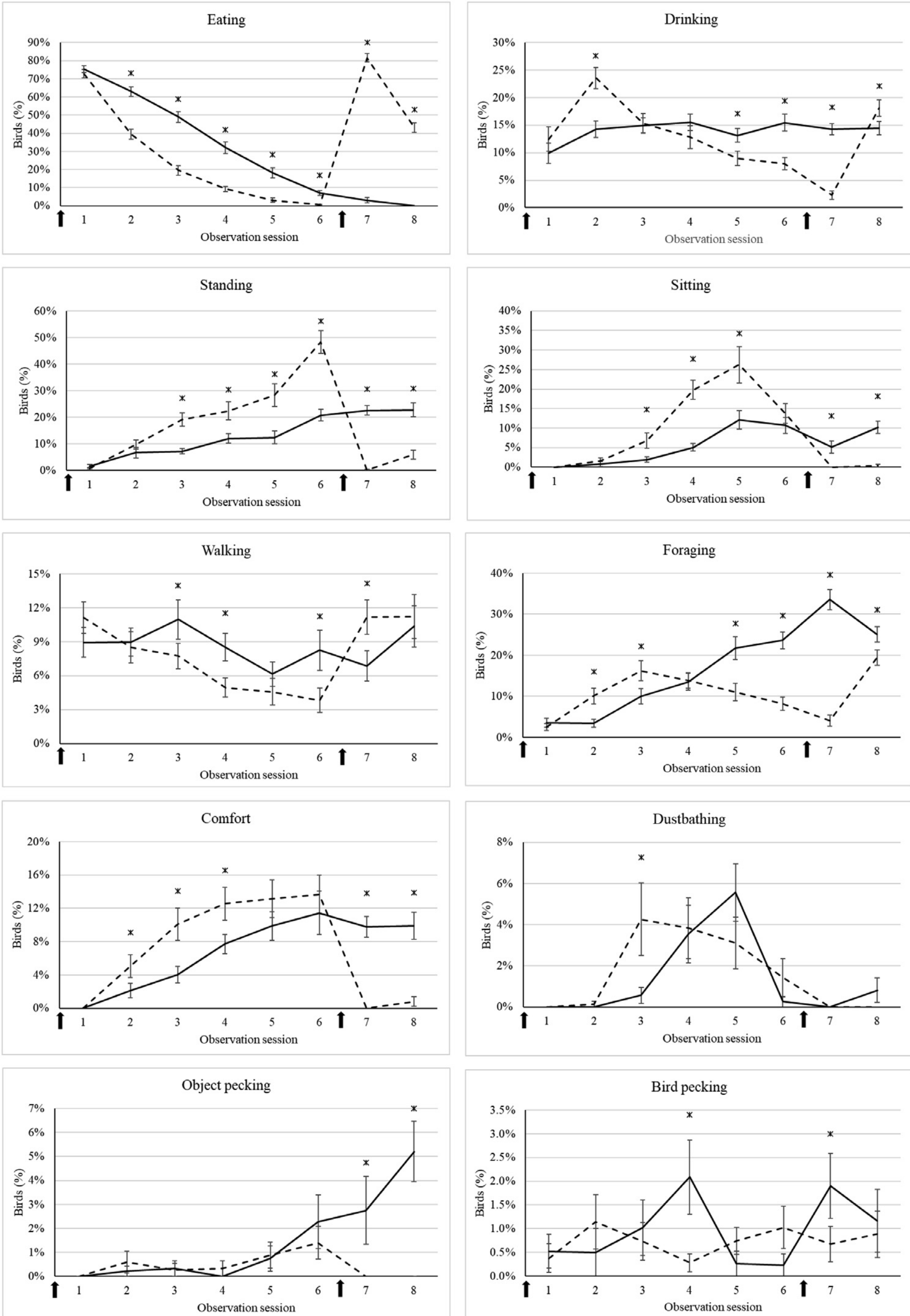

Figure 1. Effect of feeding strategies on the development of behavior over the eight observation sessions. Arrows indicate moment of feeding (1st: 0730 h and 2nd: 1400 h). Solid line = birds fed once a day and dashed line = birds fed twice a day. Error depict the standard error of the mean (SEM). Asterisks indicate significant differences ($P \leq 0.05$) between treatments.

fasting period reduced gonadotropin secretion from the hypothalamus, which negatively affects the hormonal pathways involved in ovulation, and resulted in inferior egg production. However, this merits further study.

Split feeding fed birds tended to a higher egg production between 51 and 55 wk of age compared to the birds fed once a day the control diet, resulting in 1.1 more eggs per bird. Nevertheless, no significant effect was observed for the second half of the experimental period. No comparable studies with breeders and split feeding are known to the authors, however, some information from layers is available. Studies with split feeding with layers in aviary systems (Molnár et al., 2018) and organic layers (van Krimpen et al., 2018) showed no differences in egg production.

No effect of twice a day feeding on egg weight was observed, which is in agreement with studies by Lewis and Perry (1988), Samara et al. (1996), Backhouse and Gous (2005), Soltanmoradi et al. (2013), and Londero et al. (2015, 2016). On the other hand, Cave (1981), Spradley et al. (2008), Taherkhani et al. (2010), and Moradi et al. (2013) found higher egg weights when breeders were fed twice a day. The inconsistency in results in the literature might be caused by differences in length of the experimental period. In the experiments of Lewis and Perry (1988), Samara et al. (1996), Soltanmoradi et al. (2013), Londero et al. (2015, 2016), and the present study, twice a day feeding was applied during a period between 5 and 12 wk. In the studies of Cave (1981) and Spradley et al. (2008), who found an increased egg weight, twice a day feeding was applied during the entire laying period.

In the present experiment, it was expected that the split feeding birds would produce lower egg weights, but this was not observed. The average content of the ingredients influencing egg size (methionine, cysteine, and linoleic acid) of the morning and afternoon diets were lower for the split feeding compared to the control diet, as calculated. Total methionine and cysteine was 4.3% lower (4.96 vs. 5.18 g/kg) and linoleic acid was 6.7% lower (15.4 vs. 16.5 g/kg). However, the analyzed crude protein content was 0.5% higher (138.5 vs. 138.0 g/kg) in the split feeding compared to the control diet, which may explain why no effects of split feeding on egg weight were found.

Effect of Feeding Strategy on Eggshell Quality

Eggshell quality was not affected by twice a day feeding or split feeding, which is in line with studies of Samara et al. (1996), Backhouse and Gous (2005), Spradley et al. (2008) and Londero et al. (2015, 2016). On the other hand, Lewis and Perry (1988) and Soltanmoradi et al. (2013) found a higher eggshell weight when breeders were fed twice compared to once a day. A higher eggshell weight was also found by de Los Mozos and Sanchez, 2014 in old laying hens (95−98 WOA) fed a split feeding diet. The lack of a positive effect on eggshell quality by applying twice a day feeding and split feeding in the present study is probably caused by the weekly provision of additional oyster shells in the afternoon for all birds. It is, therefore, suggested that the extra (coarse) calcium source reduced possible effects on eggshell quality of the treatments. Even though the contrast in calcium supply remained the same between treatments, since all treatments received the same amount of oyster shells, it is possible that calcium was provided in excess resulting in the maximum calcium level in the blood for all treatments (Bar et al., 1979).

Moreover, a combination of factors such as age of the breeders, time between the second meal and lights off, and calcium source could affect eggshell quality. Older flocks show an extended oviposition pattern (Zakaria et al., 2005; Zakaria and Omar, 2013), meaning that the next ovulation and eggshell formation also occurs later on the day, which results in a shift in nutrient requirements as well (Bootwalla et al., 1983). Large differences between the second feeding time and lights off (3−9 h) were used in previous studies. Calcium intake during the second feeding time is necessary to have the required calcium available for eggshell formation during the night (Leeson and Summers, 2005). It has been shown that 4 h after feed intake the crop contained, less than 50% of the calcium intake which result in a fast absorption of calcium (Farmer et al., 1983a). Calcium is absorbed from the duodenum and available to use in approx. 8 h (Bar et al., 1979; Farmer et al., 1983b), presuming that a shorter period between the second feeding time and lights off is maybe beneficial for calcium metabolism.

It is previously postulated that the fineness of the calcium source is important for eggshell formation (Molnár et al., 2018). Fine (<0.2 mm) and coarse (0.6 −1.2 mm) limestone sources are used in practice as a calcium supplement, however they differ in size and solubility (Leeson and Summers, 2005). Calcium in fine limestone is directly available for absorption compared to coarse limestone what becomes available more slowly (Zhang and Coon, 1997). Relative solubility of fine, coarse, or extra coarse limestone is 100, 70, and 55%, respectively (Leeson and Summers, 2005). It is therefore mentioned that more fine limestone could be used in the morning diet to support Ca reabsorption to bone, and coarse limestone in the afternoon diet to support eggshell formation during the night (Molnár et al., 2018). In the present study, no differences in fine and coarse limestone were applied between the morning and afternoon diets. The control, morning and afternoon diets contain 12, 15, and 9% fine calcium source (chalk) which is probably not optimal for calcium provision for eggshell formation.

Effect of Feeding Strategy on Incubation Traits

No effects of the feeding strategies were found on incubation traits, which is consistent with the study of Spradley et al. (2008). In contrast, Soltanmoradi et al. (2013) found that feeding breeders twice a day resulted in a higher fertility and hatchability. Furthermore, Londero et al. (2015, 2016) found no effects of feeding at 0800 h (100%), 0900 and 1500 h (50/50%), and 1500 h (100%) on fertility and hatchability. They observed, however, that birds fed the full feed portion at 0800 between 28 and 40 WOA, had a lower embryonic mortality than the birds fed twice a day or only in the afternoon (9.3 vs. 12.1 and 11.8%, respectively) which resulted in a higher hatchability of fertile eggs (Londero et al., 2015). The lower embryonic mortality of the birds fed once in the morning was caused by the lower specific gravity, eggshell weight, and thickness, which made gas exchange and moisture loss easier. In general, poor eggshell quality is not an issue in the first half of the laying period and normally only occurs in the second half of the laying period.

Effect of Feeding Strategy on Behavior

A significant effect of treatment on behavior was found, especially between once and twice a day feeding, whereas the nutritional differences (split feeding vs.

control diet twice a day) did not seem to have any influence on birds' behavior. In general, birds fed twice a day spent more time on eating and sitting and less time on foraging and object pecking. Despite the same amount of daily feed, twice a day fed birds spent more time on eating which could be caused by a calmer feed intake. This is underlined by the fact that these birds were less active between the 2 feeding times and spent more time on resting (standing and sitting) and comfort behavior during that particular period (Figure 1). It has been previously hypothesized that decreased resting, sitting, and comfort behavior in feed restricted pullets is related to a lower state of hunger and higher satiety (Hocking et al., 1996). An increase in standing behavior could also reflect an increase of anticipation of the meal, as has been shown by de Jong et al. (2003), which could indicate an increased state of hunger. In the current study, observation session 6 shows a peak in time spent on standing behavior right before the meal, which is also observed with both once and twice a day feeding in the rearing period by Mens et al. (manuscript in preparation). However, the design of the current study does not include the appropriate measurements to discriminate or further investigate the motivation behind the increase in standing behavior, as for example a novel food test could show (Nielsen et al., 2011).

No comparable behavior results from literature are available on twice a day feeding in breeders, however, some work is done with rearing birds. In a study of de Jong et al. (2005), pullets were fed twice a day via a trough or scatter feed. They found, contrary to the results from the present study, more walking behavior when pullets were fed twice a day. Differences between the previous and present study are probably caused by differences in severity of feed restriction between the rearing and laying period. Broiler breeders are fed restricted between 40 and 60% of ad libitum feed intake during the rearing period (Arrazola, 2018) and between 50 and 90% during the laying period (Bruggeman et al., 1999).

Time spent on object pecking and foraging was reduced for the birds fed twice a day compared to birds fed once a day. This is in agreement with experiments with breeder pullets (van der Haar and van Voorst, 2001; de Jong et al., 2005), though no effect was found by Mens et al. (manuscript in preparation) in breeder pullets. The absolute level of object pecking (approx. 1%) was very low, however birds fed twice a day showed 3 times lower object pecking which is an indication of less frustration in broiler breeders (e.g., Savory et al., 1996; de Jong et al., 2002). Stereotypic object pecking is especially observed during the rearing period, whereas in the laying period this phenomenon is hardly observed (Sandilands et al., 2005; de Jong et al., 2005; van Emous et al., 2015), likely due to the much higher amount of feed that is provided during lay.

It is suggested that the lower level of time spent on object pecking in birds fed twice a day in the present study was caused by feeding the birds a second time. This is underlined by the increased time spent on object pecking performed by the once a day fed birds in the afternoon (Figure 1). Due to the opportunity to spent a second time eating, the time budget of the birds was adjusted, as birds fed twice a day spent almost 3% more of their time on eating compared to the birds fed once a day. Time spent on eating cannot be spent on something else like object pecking, which agrees with results found by Mason et al. (2006) and van Emous et al. (2015). Increasing the feeding frequency adjusted the behavioral patterns around feeding, that is, standing in anticipation of feeding and comfort behavior, which probably positively influenced the need to perform object pecking. Furthermore, domestic birds spent a large proportion of their time on pecking (Dawkins, 1989), since they use their beaks to forage and explore the environment (Schütz and Jensen, 2001). Furthermore, it is hypothesized that due to a better distribution of feed intake throughout the day, the feed is more evenly present in the gastrointestinal tract, possibly making the birds feel more saturated. Satiety could reduce the need to peck, since pecking is part of the birds' (normal) behavioral repertoire to find and ingest feed (Hetland et al., 2004). The results thus suggest that feeding twice a day results in more favorable behavior, although it remains to be determined whether indeed saturation is improved.

Behavioral patterns during the observation days were clearly influenced by twice a day feeding. The graphs show that birds fed once a day spent less time on eating and drinking in the afternoon. This was expected since the once a day fed birds received the total amount of feed in the morning, which was consumed within 6 h. The birds that were fed twice a day spent relatively less time in the morning on eating and drinking because they received only 50% of the daily portion in the morning. In the afternoon, there was a significant increase in eating and drinking behavior after the second feeding at 1400 h. The figures also show that the birds that were fed twice a day, spent more time on resting (standing and sitting) around noon before the second feeding time. The birds fed once a day showed more resting behavior at the end of the day which can potentially be detrimental to mating behavior and fertilization of the hatching eggs since the majority of mating takes place at the end of the day (Harris et al., 1980; Bilcik and Estevez, 2005). The last 3 to 4 hours of the day is biologically and physiologically the optimal moment for egg fertilization (Løvlie and Pizzari, 2007). More activity and especially mixing of the females and males in the afternoon leads to more mating activity and thus a good fertility persistent while birds aging (van Emous, 2010). Although more activity, as has been observed in twice a day fed birds, could positively influence egg fertilization, the incubation traits researched in this study did not support this hypothesis, since there were no differences in fertility.

CONCLUSIONS

The results of the present study show that twice a day feeding and split feeding during the late production period affects production performance and behavior in broiler breeders. Compared to breeders fed once a day the control diet, split feeding breeders showed a tendency to a higher egg production between 51 and 55 WOA whereas this effect disappeared to the end of the experimental period. Feeding breeders twice a day affected behavior and moreover the behavioral pattern. With feeding twice a day, eating and drinking were more spread over the day, and resting was more prominent around noon, as compared to feeding once a day. In conclusion, twice a day feeding improves behavior and split feeding improves egg production and behavior in broiler breeders, however, no effects were observed on eggshell quality and incubation traits.

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DISCLOSURES

The authors declare no conflicts of interest.

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