

# The effect of alternative feeding strategies for broiler breeder pullets: 1. Welfare and performance during rearing

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**ABSTRACT** Broiler breeders have impaired reproductive performance when fed to satiety but they can achieve an optimal hatching egg production under feed restriction. Feed restriction is a welfare concern due to signs of hunger, lack of satiety, and frustrated feeding motivation. The objective of this research was to examine the effect of a rationed alternative diet and non-daily feeding schedules on the performance and welfare of broiler breeder pullets reared under simulated commercial conditions. At 3 wk of age, 1,680 Ross 308 pullets were allocated to 24 pens fed with 1 of 4 treatments: 1) daily control diet (control); 2) daily alternative diet (40% soybean hulls and 1 to 5% calcium propionate); 3) 4/3 control diet (4 on-feed days per week; 3 non-consecutive off-feed days per week); and 4) graduated control diet (feeding frequency varied with age). Body weight and body weight uniformity were recorded at 3, 5, 7, 11, 17, and 21 wk of age. Pullets were scored

for feather coverage, foot lesions, and hock burns bi-weekly. Physiological indicators (plasma glucose, corticosterone, hematology, and feather traits) and feeding motivation were also determined throughout rearing during on- and off-feed days. Data were analyzed using a linear mixed regression model, with pen nested in the model and age as a repeated measure. Compared to control, pullets under the 3 alternative feeding strategies had a lower feeding motivation during early rearing ( $P = 0.03$ ), better feather coverage throughout rearing ( $P = 0.001$ ), fewer feather fault bars ( $P = 0.006$ ), and a delayed increase in the basophil to lymphocyte ratio ( $P = 0.001$ ). These results indicate that the 3 alternative feeding strategies (the alternative, the graduated, and the 4/3 schedule) may decrease feeding motivation and alleviate stress compared to the control, suggesting an overall improvement in broiler breeder welfare without negative consequences on their performance.

**Key words:** stress, feeding motivation, rearing feeding strategy, alternative diet, non-daily feeding

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## INTRODUCTION

Broiler breeders share the same genetic potential for fast growth and high feed intake as their progeny (Hocking et al., 1997; Bokkers and Koene, 2003; van Krimpen and de Jong, 2014). However, feeding broiler breeders to satiety can lead to obesity-related problems (Hocking et al., 2002; D'Eath et al., 2009; van Krimpen and de Jong, 2014). Therefore, broiler breeders are restricted during rearing to reduce health problems and to achieve an optimal reproductive per-

formance during lay (Gous et al., 1999; D'Eath et al., 2009). Pullets are restricted to approximately 43% of ad libitum feed intake for the same body weight of broilers during rearing. This chronic feed restriction results in hunger, lack of satiety, and feeding frustration (D'Eath et al., 2009; van Krimpen and de Jong, 2014) as indicated by behavioral signs, feeding motivation tests, and stress indicators (Savory and Maros, 1993; Savory et al., 1996; de Jong et al., 2002, 2003). Feed restriction in broiler breeders often leads to gentle feather pecking redirected towards the tail and vent leading to a decreased feather coverage in broiler breeders (Nielsen et al., 2011; van Emous et al., 2015). The feather coverage score is considered a welfare indicator that is positively correlated with overall body feather weight (Leeson and Morrison, 1978) and relates to feather pecking activity (Girard et al., 2017a), both gentle feather pecking and severe pecking (de Jong et al., 2013; Morrissey et al., 2014a). In addition, feather traits, such as feather growth and fault bars, can be used as an indirect measure of nutritional

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status, body condition, and stress (Riddle, 1908; Murphy et al., 1988; Romero et al., 2005; Stochlic and Romero, 2008; DesRoches et al., 2009; Arrazola and Torrey, 2019). For this reason, the indicators of feeding motivation such as feeding motivation test, stress response, feather coverage score, and feather traits are useful indicators to assess the welfare of broiler breeders under feed restriction.

Feed restriction in broiler breeders is a welfare concern in the meat poultry industry due to the remaining high feeding motivation (D'Eath et al., 2009; van Krimpen and de Jong, 2014). Thus, the development of alternative feeding strategies has focused on promoting satiety and reducing hunger by increasing feed allotment (g) either by diluting caloric content (qualitative restriction) or by reducing feeding days per week in exchange for larger feed allotment during on-feed days (non-daily quantitative feed restriction). Alternative diets used for qualitative restriction include dietary caloric diluents, appetite suppressants, or the combination of both (Sandilands et al., 2005; van Emous et al., 2015). When fed ad libitum, these alternative diets could not limit body weight gain (Savory et al., 1996; Sandilands et al., 2006) and resulted in low body weight uniformity (Savory et al., 1996). When rationed, however, alternative diets used under experimental conditions may improve broiler breeder welfare and help achieve performance objectives, allowing a larger feed allotment and a longer feeding duration (Bennett and Leeson, 1989; Savory et al., 1993; Nielsen et al., 2011; Morrissey et al., 2014a,b; Zuidhof et al., 2015). Nevertheless, whether the benefits for broiler breeder welfare hold true under commercial conditions remains unclear. Another feeding strategy is non-daily quantitative feed restriction, commonly used in North America, to allow a more even feed distribution resulting in an improvement in body weight uniformity and lower feeding competition (Zuidhof et al., 2015). Non-daily feeding schedules eliminate one or more non-consecutive feeding days per week in exchange for feeding a larger feed allotment during on-feed days (de Beer and Coon, 2007, 2009; Morrissey et al., 2014b). However, non-daily feeding schedules are banned in some countries because of the perceived insult to welfare of not having a daily meal (DEFRA, 2007). Nonetheless, there is yet a lack of scientific evidence that daily feed restriction promotes better broiler breeder welfare compared to non-daily feed restriction.

For this reason, the objective of this research was to examine the effect of a rationed alternative diet and non-daily feeding schedules on the performance (growth rate and body weight uniformity) and welfare indicators (health, stress indicators, and feeding motivation) of broiler breeder pullets under simulated commercial conditions. Based on previous studies (de Jong et al., 2005; Sandilands et al., 2005; Morrissey et al., 2014a; Zuidhof et al., 2015), the rationed alternative diet was hypothesized to improve body weight uniformity, decrease feeding motivation, and lower the stress response

compared to the control diet, either fed daily or non-daily. Non-daily feeding was predicted to slow growth rate, improve body weight uniformity, and improve feather coverage compared to daily control diet but to increase the stress response as suggested in de Beer and Coon (2007), Morrissey et al. (2014a), and Zuidhof et al. (2015).

## MATERIALS AND METHODS

A total of 1,750 Ross 308 broiler breeder pullets were housed at the Arkell Poultry Research Station (Guelph, ON, Canada) at 1 D of age from February 2015 to July 2015. All the procedures used in this experiment were approved by the University of Guelph's Animal Care Committee (AUP # 3141) and were in accordance with the guidelines outlined by the Canadian Council for Animal Care (NFACC, 2016).

### *Housing and Management*

Chicks were donated courtesy of Aviagen (via Horizon Poultry, Hanover, Ontario, Canada) and were vaccinated based on local recommendations and the health program in the research facility. Chicks were beak treated using infrared beam at the hatchery. Upon arrival, chicks were randomly allocated to 24 floor pens (16.9 chicks/m<sup>2</sup>) until 20 D of age. Floor pens (2.36 m wide × 1.83 m deep) included nipple drinker lines providing water ad libitum. Room temperature started at 32°C at 1 day old and gradually decreased to 29°C by 2 wk of age. The relative humidity was 21 ± 1% during the same period. The light program started at 70 lux for 23L:1D and switched to 21 lux for 12L:12D at 4 D of age until 21 D of age. A starter diet was fed ad libitum during the first week of age and feed-restriction starting at 8 D of age based on breed recommendations (Aviagen, 2011).

At 3 wk of age, 1,680 pullets were selected based on body weight and housed in 24 floor pens of 70 pullets per pen (7.7 pullets/m<sup>2</sup>) from 3 to 22 wk of age. Floor pens (3.7 m wide × 2.5 m deep × 4 m high) were visually isolated from neighbor pens with plastic white walls to 1.2 m high. Pens were equipped with 2 drinker lines (14 nipples, 1 nipple per 5 pullets), 2 feeders, and 2 perches. Water was provided ad libitum, and trough feeders (13 cm wide × 152 cm long × 5 cm deep) with male exclusion grills were hung at 10 cm above the litter. Feeder space was fixed at 8.7 cm/pullet during rearing. Folded slats provided 3.3 m<sup>2</sup> perching surface 0.45 m above substrate (3.7 m wide and 0.6 m deep). Bedding was 5 cm deep pine wood shavings, and litter was not replaced during the rearing phase.

Management practices were in accordance with breeding company recommendations (Aviagen, 2013a) and consistent across treatments. Room temperature was controlled by air and ground heating systems, and

**Table 1.** Experimental composition of the control and the alternative diets in starter (3 to 6 wk of age), grower 1 (7 to 12 wk of age), and grower 2 (13 to 22 wk of age).

Ingredient	Control			Alternative <sup>1</sup>		
	Starter	Grower 1	Grower 2	Starter	Grower 1	Grower 2
Corn (%)	60.6	60.8	60.8	35.8	36	35.2
Wheat shorts (%)	8.8	16.0	16.0	5.2	8.8	8.1
Soybean meal (%)	24.0	16.5	16.5	14.2	9.8	9.6
Limestone (%)	3.1	3.1	3.1	1.3	0.6	–
Dicalcium phosphate (%)	2.0	2.1	2.1	1.2	1.2	1.2
Salt (%)	0.34	0.36	0.36	0.2	0.2	0.2
Vitamin-mineral premix (%)	1.0	1.0	1.0	0.6	0.6	0.6
Methionine (%)	0.12	0.11	0.11	0.07	0.06	0.06
Calcium propionate (%)	–	–	–	1.44	3.19	5.05
Soybean hulls (%)	–	–	–	40.0	39.5	40.0
Analyzed feed composition <sup>2</sup>						
AME <sup>3</sup> (Mcal/kg)	2.53	2.48	2.48	1.68	1.68	1.69
Ethanol soluble (%)	3.70	4.86	3.62	1.77	1.62	1.92
Crude protein (%; N x 6.25)	16.13	14.46	14.84	13.65	13.82	13.49
Ca:P ratio	1.94	1.78	1.76	2.52	2.45	2.59
Calcium (%)	1.54	1.61	1.55	1.27	1.23	1.31
Phosphorus (%)	0.79	0.91	0.88	0.50	0.50	0.50
Sodium (%)	0.15	0.17	0.17	0.11	0.11	0.10
Potassium (%)	0.75	0.76	0.77	0.85	0.86	0.85
Magnesium (%)	0.18	0.22	0.22	0.21	0.21	0.21
Crude fat (%)	2.50	2.44	2.43	1.76	1.75	1.77
Starch (%)	40.39	40.09	40.65	24.53	24.43	24.64

<sup>1</sup>Feed allotment was 40, 50, and 54% higher in starter, grower 1, and grower 2 diets, respectively, than control diet.

<sup>2</sup>Analyzed at Agri-Food Laboratories, Guelph, ON, Canada.

<sup>3</sup>Apparent metabolizable energy.

ground heaters were activated from 5 to 12 wk of age in all rooms to maintain room temperature. Room temperature decreased from 27°C at 3 wk of age to 21°C by 6 wk of age. The relative humidity increased from 41 to 72% as pullets aged. The light program decreased to 21 lux for 8L:16D at 22 D of age and again to 15 lux at 56 D of age due to feather pecking. At 21 wk of age, light intensity increased to 54 lux. Lights came on at 8:00 am and pullets were fed approximately 30 min later.

Feed allotment was calculated according to Aviagen (2011) and adjusted for mortality twice per week. From 3 to 22 wk of age, pullets were fed 1 of 4 feeding strategies (i.e., treatments): (1) control diet fed daily (control), (2) alternative diet fed daily, (3) control diet fed on a 4/3 schedule, and (4) control diet fed on a graduated schedule. Diets were fed crumble pellets using a starter diet (small crumble) from 0 to 6 wk of age, Grower 1 (medium crumble) from 7 to 12 wk of age and grower 2 (medium crumble) from 13 to 22 wk of age (Table 1). Mortality was recorded as it occurred.

## Experimental Design

The experimental design for the rearing phase was a randomized block design with 4 treatments and 6 replicates per treatment. Pens were allocated to 4 rooms and all treatments were represented in each room. The experimental design controlled for location within room (side, location, and neighbor treatment).

The control diet was formulated according to nutritional specifications (Aviagen, 2013b), and the alter-

**Table 2.** Weekly feeding schedule for pullets fed on the graduated treatment, indicating off-feed days (×) and on-feed days (√).

Age (week)	Feeding schedule	Sun.	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.
3–4	5/2	√	×	√	√	×	√	√
5–11	4/3 <sup>1</sup>	√	×	√	×	√	×	√
12–18	5/2	√	×	√	√	×	√	√
19–22	Daily	√	√	√	√	√	√	√

<sup>1</sup>Pullets on the fixed 4/3 feeding schedule followed the same weekly feeding schedule.

native diet was a dilution of the control diet with 40% soybean hulls at a fixed inclusion rate and with calcium propionate at 1.44, 3.19, and 5.05% in starter, grower 1, and grower 2 diets, respectively (Table 1). Control pullets were fed a control diet daily; pullets on the 4/3 and the graduated schedule were fed the control diet on a non-daily feeding schedule. Pullets on the 4/3 schedule were fed 4 days per week (on-feed days), with 3 non-consecutive off-feed days per week. Pullets on the graduated schedule were fed on a varying feeding frequency based on pullets' age (Table 2). All treatments provided the same apparent metabolizable energy per week. Pullets on the alternative diet received an increased daily feed allotment of 40% starter, 50% grower 1, and 54% grower 2. Pullets on the 4/3 and 5/2 schedules received an increased in feed allotment of 75 and 40%, respectively, during on-feed days. Diets were formulated to be iso-nutrient for the same caloric allotment, but the proportions of crude protein and calcium were 4.46 and 0.56%, respectively, higher for the alternative diet than the control diet throughout rearing (Table 1).

**Table 3.** Feather coverage scoring system for each of the 6 body parts integrating the percentage of feather coverage loss and skin lesion of each body area.<sup>1</sup>

Score	Feather coverage
0	Completely covered
1	>10% of feathers missing, wet or broken feathers
2	10 to 50% of feathers missing, wet or broken feathers (no blood or tissue damage)
3	>50% of feathers missing, wet or broken feathers (no blood or tissue damage)
4	10 to 50% of feathers missing, wet or broken feathers including tissue damage
5	>50% of feathers missing, wet or broken feathers including tissue damage

Scores from the 6 areas were summed together to result in a total body feather coverage score from 0 to 30, with 30 being the worst feather coverage.

<sup>1</sup>Morrissey et al. (2014a).

## Data Collection

**Body Weight and Body Weight Uniformity** A subsample of randomly selected 25 pullets per pen was weighed at 3, 6, 10, 14, and 17 wk of age to examine growth rate and body weight uniformity. Body weight was measured on on-feed days after consumption of their daily feed allotment. Body weight uniformity was determined based on the coefficient of variation (CV) and calculated by dividing the standard deviation per pen by the average body weight per pen.

At 22 wk of age, all pullets were weighed and assessed for sexual maturity by 2 trained observers using a visual yes/no score (Aviagen, 2013a). Pullets were considered *mature* if comb or wattles were redder than the rest of the caruncles. Interobserver reliability was determined at 20 wk of age for a subsample of 70 pullets. Each observer independently scored the same subsample of pullets, and the percentage of agreement between observers was 98.6% ( $\kappa = 0.94$ ) for the maturity score.

**Foot Lesions, Hock Burns, and Feather Coverage** A subsample of 10 pullets per pen was individually wing-tagged (Ketchum Mfg. Co. Inc., Lake Luzerne, NY) and dye-identified on wing plumage (concentrated pink gel colors, Wilton Industries, Woodridge, IL). These pullets were scored biweekly (i.e., every other week) for foot lesions, hock burns, and feather coverage. The presence of foot lesions and hock burns was recorded using a yes/no basis. *Foot lesions* and *hock burns* were defined as tissue damage, bleeding, coagulated injuries, inflammation, necrosis, and/or hematoma of the foot pad(s) and hock(s), respectively. Both feet and legs were scored including foot and toe pad. The presence of foot lesions or hock burns was separately calculated per pen by dividing the number of pullets with lesions/hock burns by the number of pullets scored.

Body feather coverage was scored based on Morrissey et al. (2014a), as shown in Table 3. Six body parts (including head, neck, back, wing, vent, and tail) were independently scored from 0 to 5. Individual scores for each body part were summed together to result in an overall maximum body feather coverage score of 30 per pullet. High scores indicate poor feather coverage and the presence of skin lesion(s). *Skin lesions* refer to tissue damage, bleeding, and coagulated injuries

and hematoma. Bleeding follicles fell within this definition. The presence of skin lesions was also recorded in a yes/no basis and reported as the percentage of pullets with skin lesions divided by the number of pullets scored.

Two observers scored pullets for the presence of foot lesion(s) and hock burn(s) and feather coverage condition, and each observer scored the same number of pullets per pen and week. Interobserver reliability was determined at 12 wk of age for a subsample of 60 pullets. Each observer independently scored the same subsample of 5 randomly selected pullets per pen and 12 pens (3 pens per treatment). The percentage of agreement between observers was 98.2% ( $\kappa = 0.79$ ), 96.5% ( $\kappa = 0.49$ ), and 77.2% ( $\kappa = 0.71$ ) for the prevalence of foot lesions, hock burns, and feather coverage score, respectively.

**Feather Traits** From the previous subsample of pullets scored for foot lesions, hock burns, and feather coverage, 2 wing feathers per pullet were collected from the same follicle at 15 (juvenile feather) and 21 wk of age (adult feather). The primary 8 (P8, the third outermost wing feather) was selected as representative of the wing feathers. From the right wing, the wing feather was plucked at 15 wk of age (juvenile feather) during the juvenile-to-adult molt. The removal of the juvenile feather induced the regrowth of a new feather in a synchronized manner to replace the previous one, referred to here as the *induced feather*. The induced feather started to regrow at 17 wk of age. During regrowth, reference marks were drawn on the rachis of the feather using a permanent marker at 19 and 21 wk of age to quantify biweekly growth rate. At 22 wk of age, the induced feather was cut without damaging the growing follicle. Both feathers were analyzed for the prevalence of fault bars, feather weight, and length by an observer blind to treatments. Fault bars were macroscopically identified against light and categorized according to their length and severity as moderate (<5 mm) and severe ( $\geq 5$  mm). A milligram scale (Ohaus E01140 nearest at 0.1 mg) was used to weigh feathers, and a digital caliper (Mitutoyo Absolute Digimatic calipers nearest at 0.01 mm) was used to measure feather length. The number of fault bars was counted per feather, and feather growth was estimated for the induced feather by dividing its length and weight

by the number of days between reference marks. Broken and very dirty feathers were excluded.

**Physiological Measures** Six out of the ten pullets per pen scored for foot, hocks, and feather coverage condition were selected for repeated blood collection at 5, 11, 18, and 21 wk of age: 3 pullets on on-feed days and another 3 on off-feed days. The order of feeding day alternated depending on the week to avoid predictability of the blood collection. Blood samples were collected after pullets consumed their daily feed allotment and sampling time after being fed was recorded (between 9:00 am and 2:00 pm). Whole blood (2 mL per pullet) was collected within 3 min of the pullet being caught (Morton, 1993). Whole blood was collected with syringes and transferred into EDTA vacutainers, and samples were placed in racks, avoiding direct contact with ice within a portable cooler. Before centrifugation, blood samples were carefully mixed for blood smears. Vacutainers remained in the cooler for a few hours after blood collection until centrifugation. Samples were centrifuged at 1,747 *g* (2,500 rpm) for 15 min at 4°C. Blood smears and plasma samples were blindly coded with the individual wing tag and a random number for the collection day. Plasma samples were frozen at -20°C in duplicate and analyzed after all plasma samples were collected at 21 wk of age.

Blood smears were dried for 24 h at room temperature in a smear box and smears were dyed using a modified Wright's stain (Hematek stain PAK, Siemens). The number of heterophils and basophils was counted per 100 lymphocytes per sample at 100× magnification. Samples reporting values outside physiological cut-off values for broiler breeders given by Wakenell (2011) were repeated due to intra-smear variation (Lindholm et al., 2018). Four observers were trained to identify the phenotype of 6 white blood cells for meat-type chickens (lymphocytes, heterophils, basophils, eosinophils, monocytes, and thrombocytes) according to Wakenell (2011). Observer effect was considered to avoid confounding amongst week, feeding day, and treatment. Data collection started once observers achieved ≥90% interobserver reliability. The heterophil/lymphocyte (**H/L**) and basophil/lymphocyte (**B/L**) ratios were determined by dividing the number of heterophils and basophils, respectively, by the number of lymphocytes (approx. 100). The percentage of agreement between observers was 95.8% ( $\kappa = 0.93$ ) at identifying white blood cells (lymphocytes, thrombocytes, heterophils, basophils, monocytes, and eosinophils). Plasma samples were defrosted in a refrigerator 1 h before corticosterone (**CORT**) extraction and 6 h before plasma glucose concentration analysis. Free plasma CORT was extracted following a cold ethanol plasma extraction (Graham et al., 2001). Free plasma CORT was estimated from plasma extracts using a corticosterone enzyme immunoassays chicken CORT ELISA Kit (MyBioSource, San Diego). Plasma glucose concentration was determined using an enzymatic Glucose (**GO**) Assay Kit (Sigma-Aldrich, Oakville, Canada). Absorbance

was read at 540 nm with a PowerWave XS Microplate Spectrophotometer (BioTek, Winooski).

**Feeding Motivation: Feed Intake Test** A different subsample of 10 pullets per pen was individually wing-tagged and dye-identified (concentrated blue gel colors, Wilton Industries) to quantify feeding motivation by measuring the feed intake relative to body weight. The feeding motivation test was performed on 2 consecutive days (5 pullets on on-feed days and a different 5 pullets on off-feed days) at 4, 8, 12, 16, and 20 wk of age. Feeding day alternated depending on week of age to avoid predictability. The 5 pullets per pen were removed from their home pen before being fed at 8:00 am and placed into additional floor pens (80 cm deep × 80 cm wide × 60 cm high). Floor pens remained beside their home pen, visually isolated with white walls and contained wood shavings and round feeders (16.3 cm per pullet during testing). Each testing week, the feeding motivation of the 5 pullets was tested as a group. Feeders were filled with 1 kg of the pullets' home diet and pullets were fed ad libitum for 20 min, whereas the pullets' home pen was fed simultaneously. Pullets and feeders were weighed before and after the feed intake test to determine body weight gain and feed consumption, correspondingly. Pullets returned to their home pen afterwards. The relative feed intake was calculated by dividing feed intake in 20 min (g) by initial body weight (g).

**Litter Moisture** Four random litter subsamples per pen were collected weekly, at least 20 cm away from the wall and avoiding the area beneath drinkers, and pooled in a representative sample per pen that ranged from 100 to 150 g. After collection, samples were dehydrated at 60°C for 24 h to estimate litter moisture. The percentage of litter moisture was calculated by dividing sample weight loss (initial sample weight subtracted of final weight after dehydration) by initial sample weight times 100.

## Statistical Analyses

The effect of rearing feeding strategies on the welfare and the productivity of broiler breeder hens was analyzed using a generalized linear mixed model, with pen nested in the model as the independent experimental unit. Statistical analyses were performed using SAS Ver. 9.4 (SAS Institute, Cary, NC) with a Glimmix procedure and the significance level was set at *P*-values lower than 0.05.

Treatment, age, and their interaction were included as fixed effects for each model. Room, pen, and pen location within the room were included in the covariance structure as random effects. Feeding day and all its interaction combinations were also included as fixed factors for blood/plasma data and the relative feed intake. Age was fit into a repeated structure with pen as the subject, and treatment as the group. Initial measures (i.e., when the experiment started at 3 wk of age) for

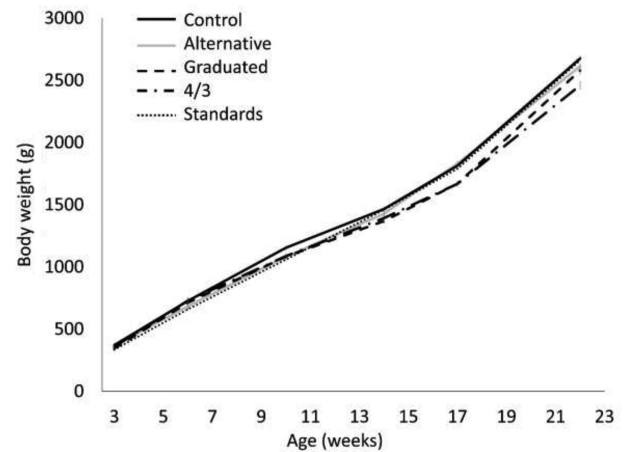
body weight, body weight uniformity, and litter moisture were integrated in the model as a covariate. Covariate factors were assessed for collinearity and excluded from the model if the variance inflation factor was higher than (or equal to) 2.5. Contrast statements were used to examine the overall effect of diet (the alternative treatment vs. the control diet [the control treatment, the 4/3 treatment and the graduated treatment]), feeding frequency (daily [the control treatment] vs. non-daily [the 4/3 treatment and the graduated treatment]), and the alternative feeding strategies [the alternative treatment, the 4/3 treatment and the graduated treatment] vs. the control treatment). Pairwise comparisons between treatments were adjusted for multiple comparisons using the Tukey test. Orthogonal regressions analyzed the effect of age into a linear, quadratic, cubic, and lack of fit response. Model assumptions were assessed using a scatterplot of studentized residuals, linear predictor for linearity, and a Shapiro–Wilk test for normality. *Outliers* were defined as observations with absolute studentized residuals higher than 3.4 and excluded from the model. The Gaussian distribution was used as the default distribution, but data were transformed if the assumption for linearity was not met.

## RESULTS

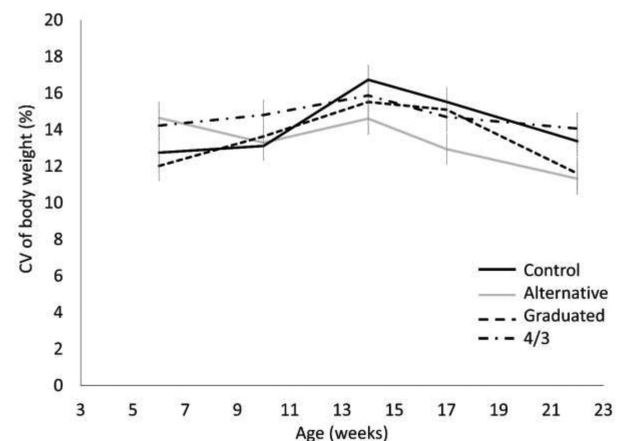
No adjustment in feed allotment was required during rearing to maintain target body weight, even when starter, grower 1, and grower 2 included 10.7, 12.9, and 12.9%, respectively, less apparent metabolizable energy than nutrition specifications (Aviagen, 2013b). Mortality remained below 1.70% from 3 to 22 wk of age without a treatment difference in the total mortality. Data are presented using estimated mean values followed by the standard error of the mean.

### Body Weight and Body Weight Uniformity

Pullets fed daily (combination of the control and the alternative diet) followed a similar growth rate throughout rearing (Figure 1) but pullets fed non-daily had a slower growth rate depending on age ( $F_{12,77} = 6.73$ ,  $P < 0.001$ ). Pullets in the control treatment were heavier at 17 wk of age ( $1805.3 \pm 28.8$  g) compared to pullets on the graduated ( $1680.9 \pm 30.2$  g;  $t_{77} = 5.16$ ,  $P < 0.001$ ) and on the 4/3 schedule ( $1667.9 \pm 32.4$  g;  $t_{77} = 5.16$ ,  $P < 0.001$ ). Pullets on the 4/3 schedule remained  $120.0 \pm 28.4$  g lighter than control pullets ( $t_{77} = 4.23$ ,  $P = 0.009$ ) at 21 wk of age, but the body weight of the pullets on the graduated schedule was similar to those in the control treatment after the feeding schedule switched to a daily feeding at 19 wk of age ( $t_{77} = 3.26$ ,  $P = 0.15$ ). Immature pullets (based on comb color) were 20% below target body weight at 22 wk of age, and feeding strategy affected the percentage of mature pullets ( $F_{3,17} = 3.67$ ,  $P = 0.03$ ). The percentage of mature pullets was higher for control ( $98.3 \pm 0.8\%$ )



**Figure 1.** The effect of diet and feeding frequency on the body weight of broiler breeder pullets during rearing (mean  $\pm$  SE). The dotted black line refers to the performance objectives for Ross 308 (Aviagen, 2011). Pullets were weighed after being fed once there was no feed left in their feeder. Pullets fed non-daily (dashed lines) had a lighter body weight at 17 wk of age, pullets on the graduated schedule showed compensatory growth, while pullets on the 4/3 schedule remained lighter until 22 wk of age ( $P < 0.001$ ).



**Figure 2.** The effect of the alternative diet and feeding frequency on body weight uniformity of broiler breeder pullets during rearing (mean  $\pm$  SE). Body weight uniformity was calculated as the coefficient of variation (CV) by dividing the standard deviation by the average body weight. The CV of body weight depended on treatment and age ( $P = 0.007$ ).

compared to pullets on the 4/3 schedule ( $93.0 \pm 0.8\%$ ;  $t_{17} = 2.32$ ,  $P = 0.03$ ).

The body weight uniformity was above 10% of the CV throughout rearing, and the effect of treatment on flock body weight uniformity depended on age ( $F_{12,78} = 2.55$ ,  $P = 0.007$ ; Figure 2). The CV of body weight remained at approximately 13 to 14% during rearing for pullets fed the alternative diet and on the 4/3 schedule. The CV for body weight significantly increased from 6 to 14 wk of age for pullets fed the control ( $\Delta = +4.0 \pm 0.8\%$ ;  $t_{78} = 4.81$ ,  $P = 0.001$ ) and on the graduated schedule ( $\Delta = +3.5 \pm 0.8\%$ ;  $t_{78} = 4.18$ ,  $P = 0.01$ ). Afterwards, the CV for body weight remained constant until 22 wk of age for the control pullets ( $13.4 \pm 0.8\%$ ) but the CV for body weight decreased for the pullets

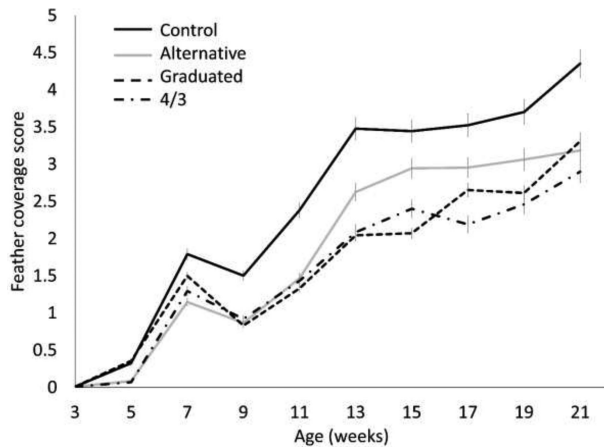
**Table 4.** The overall effect of the alternative diet and feeding frequency on health-related measures and feather coverage of broiler breeders during rearing (mean  $\pm$  SE).

	Control	Alternative	Graduated	4/3
Foot lesions (%)	4.6 $\pm$ 0.9 <sup>b</sup>	12.5 $\pm$ 2.1 <sup>a</sup>	6.2 $\pm$ 1.3 <sup>b</sup>	5.4 $\pm$ 1.4 <sup>b</sup>
Hock burns (%)	3.5 $\pm$ 0.7	5.0 $\pm$ 0.9	4.5 $\pm$ 0.5	5.2 $\pm$ 0.9
Feather coverage score <sup>1</sup>	2.4 $\pm$ 0.1 <sup>a</sup>	1.8 $\pm$ 0.1 <sup>b</sup>	1.6 $\pm$ 0.1 <sup>b</sup>	1.6 $\pm$ 0.1 <sup>b</sup>
Skin lesion (%)	1.3 $\pm$ 0.7	1.5 $\pm$ 0.8	0.5 $\pm$ 0.5	0.5 $\pm$ 0.4

Control: daily control diet; alternative: daily alternative diet; graduated: graduated schedule control diet; 4/3: 4/3 schedule control diet.

<sup>a,b</sup>Different superscripts within a row indicate significant mean differences ( $P < 0.05$ ).

<sup>1</sup>Feather coverage score out of 30, higher numbers denoting feather coverage loss and the presence of skin lesions.



**Figure 3.** The effect of diet and feeding frequency on feather coverage score (mean  $\pm$  SE). Light intensity decreased from 31 to 21 lux at 7 wk of age and increased to 54 lux at 22 wk of age. Pullets on the control treatment had a consistently higher feather coverage score than pullets on the all of the alternative feeding strategies (combination of the alternative diet, and the graduated and the 4/3 feeding schedule) ( $P = 0.001$ ).

on the graduated schedule from 14 (15.52  $\pm$  0.81%) to 22 wk of age (11.6  $\pm$  0.8%;  $t_{78} = 4.66$ ,  $P = 0.002$ ).

### Foot Lesions, Hock Burns, and Feather Coverage

Treatment influenced the prevalence of foot lesions ( $F_{3,193} = 10.73$ ,  $P = 0.001$ ), and pullets fed the alternative diet had a higher prevalence of foot lesions than those on the control diet (Table 4;  $t_{193} = 3.55$ ,  $P = 0.003$ ). Pullet's age affected the presence of foot lesions ( $F_{9,193} = 5.09$ ,  $P < 0.001$ ) and hock burns ( $F_{9,193} = 10.73$ ,  $P < 0.001$ ). The prevalence of foot lesions peaked at 7 wk of age (25.0  $\pm$  0.7%) and decreased afterwards, whereas the presence of hock burns rose until 13 wk of age (7.5  $\pm$  0.3%) and plateaued thereafter.

Neck, vent, and tail accounted for 34.3, 33.6, and 29.9%, respectively, of the feather coverage score. Feather coverage score increased consistently according to treatment ( $F_{3,240} = 16.66$ ,  $P < 0.001$ ; Figure 3) as pullets aged ( $F_{9,240} = 3.67$ ,  $P = 0.001$ ). Feather coverage score was lower for the pullets on the 3 alternative feeding strategies (i.e., combination of the alternative diet,

the 4/3 schedule and the graduated schedule) than the pullets on the control treatment ( $F_{1,240} = 47.99$ ,  $P = 0.001$ ) and the 3 pairwise comparisons were significant compared to the control (Figure 3).

### Feather Traits

Table 5 summarizes the effect of treatment on feather traits. Treatment affected the regrowth of the induced feather (weight:  $F_{3,24} = 3.43$ ,  $P = 0.03$ ; and length:  $F_{3,24} = 3.25$ ,  $P = 0.04$ ). Pullets fed non-daily (combination of the graduated and the 4/3 schedule) regrew the induced feather 0.16  $\pm$  0.04 mg/day ( $F_{1,24} = 8.24$ ,  $P < 0.01$ ) heavier and 0.21  $\pm$  0.05 mm/day longer ( $F_{1,24} = 19.39$ ,  $P < 0.001$ ) than pullets fed daily (combination of the alternative and the control treatment). From 19 to 21 wk of age, pullets on the graduated treatment regrew the induced feather 0.27  $\pm$  0.07 mm/day longer than control pullets ( $t_{24} = 3.89$ ,  $P = 0.004$ ). Treatment affected the number of fault bars ( $F_{3,48} = 4.66$ ,  $P = 0.006$ ); the number of fault bars was higher in feathers from pullets on the control treatment (4.4  $\pm$  0.4) compared to those from pullets fed the alternative diet (3.2  $\pm$  0.3;  $F_{1,48} = 2.77$ ,  $P = 0.04$ ) and on the graduated treatment (2.8  $\pm$  0.3;  $F_{1,48} = 2.77$ ,  $P = 0.04$ ). The number of fault bars did not differ between feather type ( $F_{1,20} = 0.20$ ,  $P = 0.66$ ; Table 5), but the induced feathers had a higher proportion of severe (73.8%) than moderate fault bars (26.2%).

### Physiological Measures

The effect of treatments on the H/L ratio depended on age and feeding day ( $F_{9,192} = 3.24$ ,  $P = 0.001$ ; Figure 4). The H/L ratio of pullets under the graduated schedule was higher at 11 wk of age on on-feed days (0.59  $\pm$  0.04) compared to off-feed days (0.37  $\pm$  0.04;  $t_{192} = 4.31$ ,  $P < 0.01$ ). Overall, the H/L ratio followed a quadratic curvature as pullets aged ( $F_{1,115} = 21.80$ ,  $P < 0.001$ ) with a peak at week 11 (0.52  $\pm$  0.01). The effect of treatment on the B/L ratio was impacted by age ( $F_{9,192} = 3.21$ ,  $P = 0.001$ ; Figure 5), but not feeding day ( $F_{1,192} = 1.31$ ,  $P = 0.25$ ). The B/L ratio increased significantly from 5 to 11 wk of age ( $\Delta = +0.08 \pm 0.01$ ;  $t_{192} = 4.31$ ,  $P = 0.01$ ) for control pullets, but this increase was delayed from 5 to 18 wk of age in pullets fed the alternative diet ( $\Delta = +0.07 \pm 0.02$ ;  $t_{192} = 3.96$ ,  $P = 0.01$ ), on the graduated ( $\Delta = +0.10 \pm 0.02$ ;  $t_{192} = 5.44$ ,  $P < 0.001$ ), and on the 4/3 schedule ( $\Delta = +0.12 \pm 0.02$ ;  $t_{192} = 7.02$ ,  $P < 0.001$ ).

Figure 6 illustrates that the plasma CORT concentration decreased from 5 to 21 wk of age following a logistic distribution depending on the feeding day, age, and treatment ( $F_{9,192} = 3.70$ ,  $P < 0.001$ ). During off-feed days, pullets on the non-daily treatments were fed the previous day and pullets on the control and the alternative treatments were fed on both days. At 11 wk of age, plasma CORT on the off-feed day was lower in

**Table 5.** The effect of the alternative diet and feeding frequency on the development of wing feathers before and during juvenile molting in feed-restricted broiler breeder pullets (mean  $\pm$  SE).

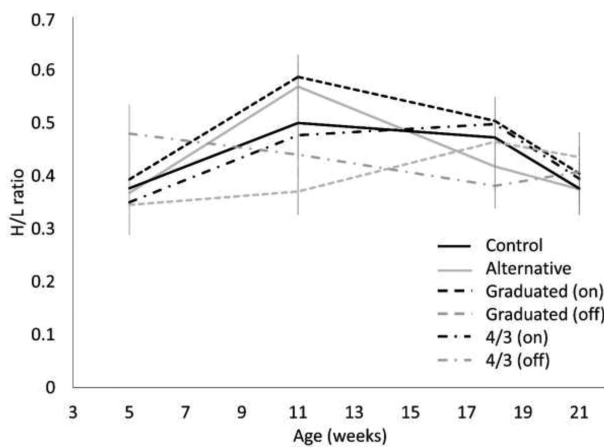
	Control	Alternative	Graduated	4/3
<i>Juvenile feather</i>				
Weight (mg)	185.59 $\pm$ 1.93	180.61 $\pm$ 2.14	186.66 $\pm$ 2.41	183.10 $\pm$ 2.36
Length (mm)	156.99 $\pm$ 1.33	156.16 $\pm$ 1.29	157.35 $\pm$ 1.32	156.57 $\pm$ 1.33
Fault bars	4.4 $\pm$ 0.6 <sup>a</sup>	2.3 $\pm$ 0.3 <sup>b</sup>	3.0 $\pm$ 0.4 <sup>a,b</sup>	3.0 $\pm$ 0.4 <sup>a,b</sup>
<i>Induced feather</i>				
Growth (mg/day) <sup>1</sup>	3.81 $\pm$ 0.04 <sup>a,b</sup>	3.63 $\pm$ 0.04 <sup>b</sup>	3.90 $\pm$ 0.04 <sup>a</sup>	3.76 $\pm$ 0.04 <sup>a,b</sup>
Growth (mm/day) <sup>1</sup>	3.07 $\pm$ 0.04	3.06 $\pm$ 0.04	3.16 $\pm$ 0.04	3.16 $\pm$ 0.04
Growth (mm/day) <sup>2</sup>	3.79 $\pm$ 0.08 <sup>c</sup>	3.87 $\pm$ 0.08 <sup>b,c</sup>	4.05 $\pm$ 0.08 <sup>a</sup>	4.04 $\pm$ 0.08 <sup>a,b</sup>
Fault bars	4.4 $\pm$ 0.8 <sup>a</sup>	3.7 $\pm$ 0.6 <sup>a,b</sup>	2.6 $\pm$ 0.4 <sup>b</sup>	3.4 $\pm$ 0.6 <sup>a,b</sup>

Control: daily control diet; alternative: daily alternative diet; graduated: graduated schedule control diet; 4/3: 4/3 schedule control diet.

<sup>a-c</sup>Different superscripts within a row indicate significant mean differences ( $P < 0.05$ ).

<sup>1</sup>From 17 to 21 wk of age (28 D).

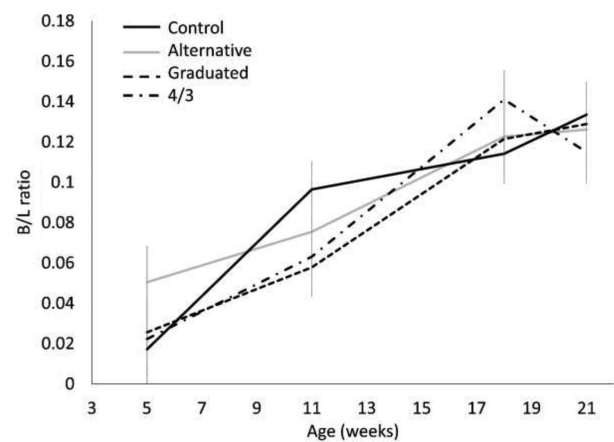
<sup>2</sup>From 19 to 21 wk of age (14 D).



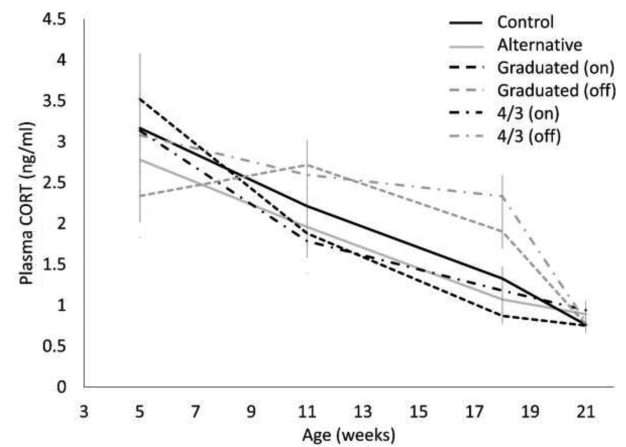
**Figure 4.** The effect of diet and feeding frequency on the heterophil to lymphocyte ratio (H/L) in broiler breeder pullets by feeding day (mean  $\pm$  SE). Solid lines represent daily-fed treatments (black for the control and gray for the alternative), and dashed lines for non-daily fed treatments (black for on-feed days and gray for lines off-feed days). During off-feed days, pullets on the 4/3 schedule and on the graduated treatment were not fed. At 11 wk of age, the H/L ratio was higher for the graduated treatment (regular dashed lines) during on-feed days compared to off-feed days ( $P = 0.001$ ).

control pullets ( $1.39 \pm 0.14$  ng/mL) compared to pullets on the graduated schedule ( $2.72 \pm 0.30$  ng/mL;  $t_{192} = 4.08$ ,  $P = 0.02$ ) and on the 4/3 schedule ( $2.60 \pm 0.28$  ng/mL;  $t_{192} = 4.02$ ,  $P = 0.03$ ). At 18 wk of age, plasma CORT was higher on off-feed days compared to on-feed days for pullets on the graduated schedule ( $\Delta = +1.29 \pm 0.36$  ng/mL;  $t_{192} = 3.95$ ,  $P = 0.02$ ) and on the 4/3 schedule ( $\Delta = +1.09 \pm 0.32$  ng/mL;  $t_{192} = 4.31$ ,  $P = 0.01$ ). The peak in plasma CORT on the off-feed day at 18 wk of age was higher in pullets on the 4/3 schedule ( $2.43 \pm 0.32$  ng/mL) compared to control pullets ( $1.28 \pm 0.35$  ng/mL;  $t_{192} = 5.29$ ,  $P < 0.001$ ).

Plasma glucose concentration was affected by treatment, feeding day, and age ( $F_{9,192} = 6.20$ ,  $P < 0.001$ ; Figure 7). At 5 wk of age, pullets on the graduated schedule had lower plasma glucose on on-feed days

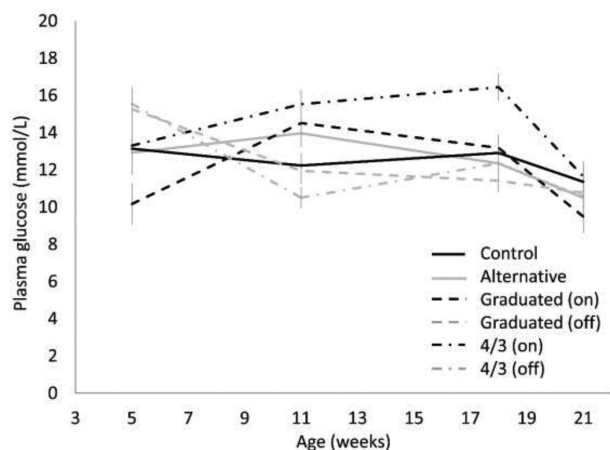


**Figure 5.** The effect of the diet and feeding frequency on the basophil to lymphocyte ratio (B/L) in broiler breeder pullets (mean  $\pm$  SE). The B/L ratio increased earlier in control (black solid line) than for the alternative feeding strategies (alternative: gray solid line, graduated: black dashed line, and the 4/3 schedule: black dashed line;  $P = 0.001$ ).



**Figure 6.** The effect of the diet and feeding frequency by feeding day on plasma CORT in broiler breeder pullets (mean  $\pm$  SE). Solid lines represent daily-fed treatments and dashed lines non-daily fed treatments (on-feed days: black, and off-feed days: dark gray). Pullets on the non-daily treatments were fed during on-feed days, and pullets fed the other treatments (solid lines) were fed on both days. Plasma corticosterone increased on off-feed days in non-daily fed treatments based on pullet's age ( $P < 0.001$ ).





**Figure 7.** The effect of the diet and feeding frequency on plasma glucose in broiler breeder days (mean  $\pm$  SE). Plasma glucose concentration was higher during on-fed days (black dashed lines) compared to off-fed days (dark-gray dashed lines) depending on feeding frequency and pullets' age ( $P < 0.001$ ).

( $10.17 \pm 1.09$  mmol/L) compared to off-fed days ( $15.27 \pm 1.03$  mmol/L;  $t_{192} = 6.28$ ,  $P < 0.001$ ), but plasma glucose concentration for pullets on 4/3 schedule remained similar regardless of feeding day ( $t_{192} = 2.66$ ,  $P = 0.69$ ). At 11 wk of age, plasma glucose for pullets on the graduated schedule was higher during on-fed days ( $14.52 \pm 0.66$  mmol/L) compared to off-fed days ( $11.95 \pm 0.69$  mmol/L). Plasma glucose was higher in pullets on the 4/3 schedule on on-fed days compared to off-fed days at 11 ( $\Delta = +4.99 \pm 0.66$  mmol/L;  $t_{192} = 7.48$ ,  $P < 0.001$ ) and 18 wk of age ( $\Delta = +4.22 \pm 0.66$  mmol/L;  $t_{192} = 6.08$ ,  $P < 0.001$ ). The glucose concentration for pullets on the 4/3 schedule during on-fed days (11 wk of age:  $2.80 \pm 0.14$  mmol/L and 18 wk of age:  $2.96 \pm 0.13$  mmol/L) was also higher than control pullets at 11 ( $2.20 \pm 0.12$  mmol/L;  $t_{192} = 4.95$ ,  $P < 0.001$ ) and 18 wk of age ( $2.33 \pm 0.11$  mmol/L;  $t_{192} = 4.86$ ,  $P = 0.001$ ), and higher than pullets fed the alternative diet at 18 wk of age ( $2.23 \pm 0.14$  mmol/L;  $t_{192} = 5.1$ ,  $P < 0.001$ ). Furthermore, plasma glucose concentration for pullets fed the 4/3 during on-fed days was  $3.27 \pm 0.83$  mmol/L and  $3.16 \pm 0.72$  mmol/L higher at weeks 5 ( $t_{192} = 4.31$ ,  $P < 0.01$ ) and 18 ( $t_{192} = 3.87$ ,  $P = 0.04$ ), respectively, than pullets on the graduated schedule.

### Feeding Motivation: Feed Intake Test

Figure 8 shows that the effect of the treatment on the relative feed intake was affected by feeding day and age ( $F_{12,178} = 1.95$ ,  $P = 0.03$ ). At 4 wk of age, pullets fed the alternative diet had a lower relative feed intake ( $3.66 \pm 0.23\%$ ) compared to those on the control treatment ( $6.8 \pm 0.22\%$ ;  $t_{178} = 11.16$ ,  $P < 0.001$ ). Moreover, the relative feed intake of pullets fed the alternative diet was consistently lower on on-fed days ( $5.21 \pm 0.20\%$ ) compared to off-fed days ( $5.95 \pm 0.17\%$ ;  $t_{178} = 4.49$ ,  $P < 0.001$ ). At 4 wk of age, the relative feed in-

take of pullets on the 4/3 schedule was lower during the on-feed day ( $\Delta = -2.00 \pm 0.42\%$ ;  $t_{178} = 4.78$ ,  $P = 0.002$ ) and the off-feed day ( $\Delta = -1.88 \pm 0.38\%$ ;  $t_{178} = 5.00$ ,  $P < 0.001$ ) compared to control pullets. The relative feed intake of pullets on the graduated schedule was only lower at 4 wk of age on the on-feed day ( $\Delta = -2.68 \pm 0.47\%$ ;  $t_{178} = 5.72$ ,  $P < 0.001$ ), but not on off-feed days ( $t_{178} = 3.35$ ,  $P = 0.27$ ), compared to control pullets. At 4 wk of age, pullets fed the alternative diet had a lower relative feed intake ( $3.27 \pm 0.34\%$ ) on on-feed days than pullets on the 4/3 schedule ( $5.24 \pm 0.34\%$ ;  $t_{178} = 4.33$ ,  $P = 0.01$ ).

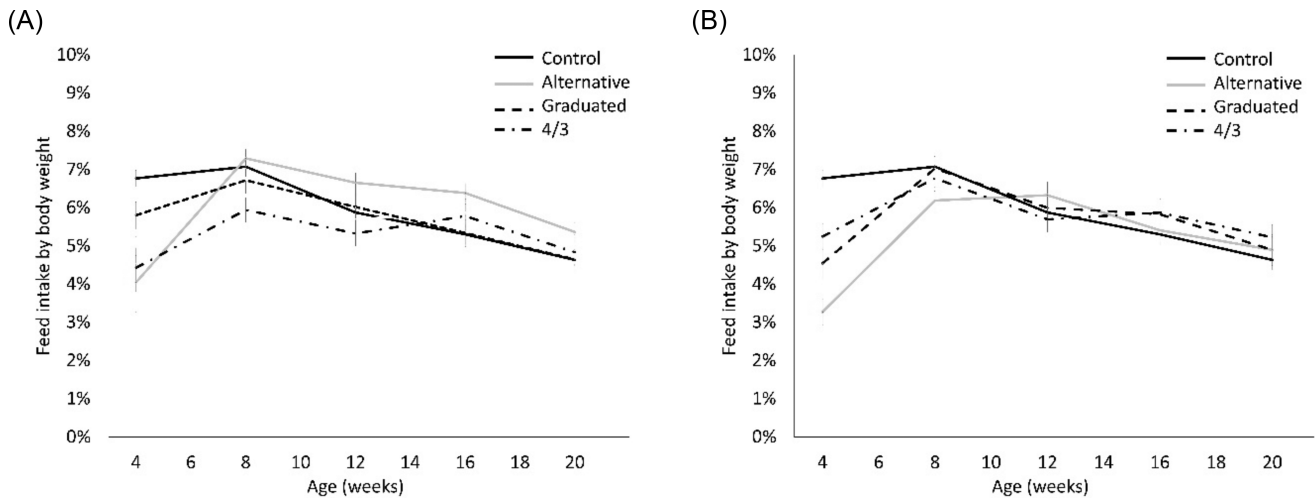
### Litter Moisture

The percentage of litter moisture was affected by treatment and pullets' age ( $F_{18,353} = 11.48$ ,  $P = 0.001$ ). The percentage of litter moisture ranged between 16.5 and 34.6% throughout rearing in the pens with pullets fed the control diet, either daily or non-daily (Figure 9), but the percentage of litter moisture in the pens with pullets fed the alternative diet increased from 4 ( $28.98 \pm 2.45\%$ ) to 6 wk of age ( $50.11 \pm 3.45\%$ ;  $t_{353} = 5.66$ ,  $P < 0.001$ ). At 8 wk of age, the difference in the percentage of litter moisture was the highest between the daily control diet ( $13.80 \pm 2.69\%$ ) and the daily alternative diet ( $47.13 \pm 3.45\%$ ;  $t_{353} = 9.49$ ,  $P < 0.001$ ). Afterwards, the percentage of litter moisture in the pens with pullets fed the alternative diet declined gradually until 12 wk of age ( $25.5 \pm 3.4\%$ ) and remained around 18% across all treatments until the 22 wk of age ( $t_{353} = 1.97$ ,  $P = 1$ ). The percentage of litter moisture affected the presence of foot lesions ( $F_{1,192} = 11.43$ ,  $P = 0.001$ ), but not the percentage of hock burns ( $F_{1,192} = 0.18$ ,  $P = 0.67$ ).

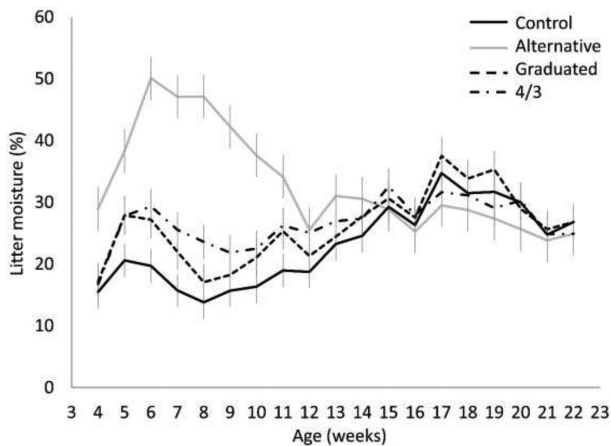
## DISCUSSION

The purpose of this study was to examine the effect of alternative feeding strategies during rearing on the performance and welfare indicators of broiler breeder pullets, and pullets fed the alternative diet were predicted to show more indicators of better welfare compared to those on the control and under the non-daily feeding strategies. Our results indicate that the pullets on the 3 alternative feeding strategies (the alternative diet, the 4/3 schedule, and the graduated schedule) showed a lower feeding motivation during early rearing, better feather coverage (lower feather coverage score) throughout rearing, and a decrease in some stress indicators (the B/L ratio and the number of fault bars) compared to the control.

Pullets on the alternative diet and non-daily feeding had a lower feeding motivation compared to control during early rearing. Previous studies indicated the lower feeding motivation under qualitative restriction on feeding motivation (e.g., using feed intake test [Sandilands et al., 2005, 2006; van Emous et al., 2015]



**Figure 8.** The effect of the diet and feeding frequency on the feeding motivation of broiler breeder pullets by feeding day (mean  $\pm$  SE). Feeding motivation is expressed as the feed intake for 20 min of ad libitum feeding relative to body weight during on-feed days (a) and off-feed days (B). Pullets on non-daily fed treatments were fed on the previous day during off-feed days and had their last meal 2 D prior to the test during on-feed days. Pullets on the daily-fed treatments (solid lines: the control in black and the alternative in gray) were both fed on the previous day. The effect of feeding strategies on the relative feed intake of broiler breeders varied based on age and feeding day ( $P = 0.03$ ).



**Figure 9.** The effect of the diet and feeding frequency on litter moisture during rearing (mean  $\pm$  SE). Ground heaters were switched on at 5 wk of age and off at 12 wk of age. Litter moisture increased in pullets fed the alternative diet (gray solid line) compared to control (black solid line) during early rearing ( $P = 0.001$ ).

and compensatory feeding [de Jong et al., 2005]), but one interesting result was the effect of the feeding day of pullets fed non-daily on the relative feed intake of pullets fed the alternative diet. Pullets on daily-fed treatments received their daily feed allotment during off-feed days and the feeding motivation test was performed 24 h after feeding for on- and off-feed days. Although a different subsample of pullets was used for the on- and off-feed days in this experiment, there was no difference in body weight and the statistical model accounted for intra- and interindividual variation. Therefore, the effect of feeding day on pullets fed daily is likely to be attributed to the feeding frequency of treatments fed non-daily. All treatments were allocated inside the same rooms and, despite visual isolation between consecutive and frontal pens, pullets were able to hear each other.

Prior to feeding on on-feed days, non-daily fed pullets may have gone up to 48 h since the last feed intake, leading to a high arousal. Previous research noted that pullets fed on a skip-a-day schedule had higher anticipatory feeding behavior activity on on-feed rather than off-feed days (Girard et al., 2017b). This hyperactivity of non-fed pullets during on-feed days might have interrupted feeding behavior during the feed intake tests in pullets fed the alternative diet, probably due to a lower feeding motivation, without an effect on the control pullets. In red crossbills (*Loxia curvirostra*), the presence of a feed-motivated neighbor had an arousal effect (physiologically and behaviorally) on the pen mate, but only if the pen mate was feed restricted too (Cornelius et al., 2010). Our results indicate a similar neighbor effect dependent on dietary dilution because pullets on the rationed alternative diet were less feed motivated than those on the daily quantitative feed restriction.

Our results indicate a slower feather coverage deterioration in pullets fed the alternative diet and on a 4/3 schedule compared to pullets on the control, in agreement with Morrissey et al. (2014a). Vent and tail areas accounted for most of the feather loss in our experiment, suggesting that the slower deterioration in feather coverage is indicative of a lower feather pecking activity compared to pullets reared on the daily quantitative feed restriction. Alternatively, feather coverage loss can also be caused by environmental features such as equipment and by nutritional status resulting in poor feather quality (van Emous et al., 2015; Campe et al., 2017). Abnormal wing feathering and consistently lower feather coverage in all body parts (neck, breast, breast, vent, back, wings, tail, and legs) were observed in pullets fed a low protein diet compared to those fed a high protein diet (van Emous et al., 2015). Our results do not indicate a detrimental effect of diet on wing feather growth. Indeed, the alternative diet resulted in lighter

wing feathers compared to the control diet, whereas the non-daily quantitative feed restriction led to faster feather growth (only in length) than daily quantitative feed restriction. Feather growth is an indicator of bird's nutritional status (Murphy et al., 1988), and our results suggest a lower nutritional condition by the rationed alternative diet than by the control diet. Under non-daily feeding schedules, the larger feed allotment during on-feed days resulted in an overall increase in feather growth despite the protein catabolism during off-feed days in contrast to daily feed restriction, probably due to higher amino acid bioavailability (de Beer et al., 2007). This observation indicates that feather growth is limited by daily quantitative feed restriction and non-daily feeding can support nutrient-dependent processes such as feather growth compared to daily qualitative and quantitative feed restriction. This finding may indicate an improved nutritional status during on-feed days for non-daily fed pullets compared to those fed daily.

Results from the feeding motivation test and the feather coverage score align with some stress indicators such as wing feather fault bars and the B/L ratio, indicating a lower stress response in pullets fed the 3 alternative feeding strategies than on the daily quantitative feed restriction. The number of fault bars was higher in the wing feathers of control pullets compared to the 3 alternative feeding strategies, and the B/L ratio increased faster during mid rearing in the control pullets compared to those on the 3 alternative feeding strategies. The formation of fault bars is an indicator of stress in avian species (Riddle, 1908; Jovani and Rohwer, 2017), although the information about fault bars in poultry is scarce (Arrazola and Torrey, 2019). These results may indicate that the 3 alternative feeding strategies reduced the experience of acute stress or reduced pullet's susceptibility to acute stress during rearing. Alternatively, previous research indicated that feathers of birds under stress (with a high number of fault bars) were less resilient to damage, especially under feed restriction (DesRoches et al., 2009), and this increase in fault bars may have weakened the plumage (contributing to a lower feather coverage score). On the other hand, previous research assessing the behavior of the broiler breeders fed the alternative and control diets indicated that pullets fed a control treatment resulted in a higher pecking activity (oral-redirected behaviors and aggressive pecking) coupled with worse feather coverage than the pullets fed the alternative treatment (Morrissey et al., 2014b). Thus, high feather pecking activity is more likely to explain differences in feather coverage, although the effect of feather quality should be considered as well. The H/L ratio is a stress indicator and has been proven to be higher under feed restriction compared to ad libitum feeding (Gross and Siegel, 1983; Hocking et al., 2001; Sandilands et al., 2006). In the current experiment, the H/L ratio was not a sensitive indicator to find differences among treatments under feed restriction in agreement with previous research (e.g., daily quantitative feed

restriction vs. qualitative restriction [de Jong et al., 2002, 2005; Sandilands et al., 2005, 2006] and daily vs. non-daily quantitative feed restriction [de Beer and Coon, 2009]), but the B/L ratio increased differentially depending on feeding strategy. Chronic feed restriction is associated with elevated B/L ratio (Hocking et al., 1996, 2001; Savory et al., 1996). Hocking et al. (1996) reported that the proportion of basophils increased with feed restriction level at 12 wk of age. Savory et al. (1996) also reported a linear increase in the proportion of basophils with feed restriction and as pullets aged. Our results indicate similar numerical values as Hocking et al. (1996) and Savory et al. (1996), suggesting that pullets on the control treatment might have experienced a higher feed restriction compared to pullets reared on the alternative feeding strategies. In our experiment, feeding day did not impact the B/L ratio but there was a difference in the H/L ratio between on- and off-feed days at 11 wk for those on the graduated schedule. At this age, the graduated treatment was fed on a 4/3 schedule, although the H/L ratio did not differ between on- and off-feed days for pullets reared on the 4/3 schedule. This difference may indicate higher physiological stress in pullets reared on the 5/2 schedule at the peak of feed restriction compared to pullets habituated to the 4/3 schedule since early rearing. Similarly, Lindholm et al. (2018) found that the H/L ratio in pullets on a 5/2 schedule was higher compared to control pullets at 12 wk of age. These results suggest that pullets on the graduated schedule might be less habituated to off-feed days at the peak of feed restriction compared to pullets on the fixed 4/3 schedule. Pullets on the fixed 4/3 schedule received a larger daily feed allotment than the graduated schedule during the early rearing period which may have enabled them to store a higher proportion of lipids for off-feed days (facilitating physiological habituation to off-feed days) than the graduated schedule. In line with this idea, de Beer and Coon (2007, 2009) reported that pullets on a 5/2 schedule were leaner at 7 wk of age than pullets on a 4/3 schedule reared at same weekly feed allotment. For this reason, pullets on the graduated treatment at 4 wk of age (when they were on a 5/2 schedule) might not have been habituated to non-daily feeding frequency due to inadequate feed allotment to meet metabolic demands for off-feed days. Meanwhile, pullets on the 4/3 schedule reduced feeding motivation up to 48 h after feeding at 4 wk of age compared to control. For this reason, pullets on the graduated schedule might not habituate to their feeding frequency as well as the pullets on the 4/3 schedule.

Similar differences between the non-daily feeding frequencies were seen in plasma CORT and glucose concentration. Previous studies found that plasma CORT increased in pullets reared at higher feed restriction levels (Savory et al., 1996; de Jong et al., 2003). However, the interpretation of plasma CORT for welfare is ambiguous as glucocorticoids are involved in the stress response as well as the regulation of glucose

homeostasis (Scanes, 2016). In our experiment, pullets fed non-daily maintained glucose concentration similar to the control during off-feed days, in agreement with de Beer et al. (2008). Previous research also suggested that broiler breeders were able to maintain constant glucose levels even after 2 consecutive off-feed days (72 h after being fed) (Belo et al., 1976; Katanbaf et al., 1989). This can be explained by a surge in free plasma CORT in pullets fed non-daily during off-feed days. During mid rearing, plasma CORT was higher in pullets fed non-daily during off-feed days compared to control. Pullets on a skip-a-day feeding had high plasma CORT on off-feed days that matched increasing concentrations of glucagon as the crop of pullets fed non-daily emptied 24 h after feeding (de Beer et al., 2007, 2008). Ralph et al. (2015) also indicated that high hepatic CORT concentration mimicked an increase in hepatic gluconeogenesis followed by a lower glycogenesis during off-feed days. Previous research and our results suggest that feed restriction higher than 50% of ad libitum feed intake (for the same body weight of broilers) might require CORT regulation for glucose homeostasis (mobilization) during off-feed days, independent of whether pullets were previously on the 4/3 or the 5/2 schedule. This conclusion is supported by Lindholm et al. (2018), where authors suggested that elevated CORT concentrations in broiler breeders is indicative of metabolic demands in regard to maintenance requirements. The effect of body weight within age in our experiment shows that CORT exponentially rose in lighter pullets at 11 and 18 wk of age, indicating the role of CORT in regulating glucose concentrations to meet nutritional demands. de Jong et al. (2003) also reported that elevated CORT coincided with an increase in feed restriction up to 30% of ad libitum feed intake (for the same age), while plasma glucose remained constant. Therefore, the welfare implications of non-daily feeding on stress physiology should be carefully examined as the increasing CORT concentrations might indicate an adaptive metabolic response to non-daily feeding and the transition from anabolism during on-feed days to catabolism during off-feed days instead of psychological stress (Scanes, 2016; Lindholm et al., 2018).

Previous results point toward chronic feed restriction being alleviated somewhat by the alternative feeding strategies used in this study, but there were limitations associated with each of them. Pullets met the target growth rate with slight differences based on feeding frequency (Aviagen, 2013b). Pullets fed non-daily were less feed efficient than those fed daily, which agrees with previous studies (de Beer and Coon, 2007, 2009; Morrissey et al., 2014b; Zuidhof et al., 2015). The slower growth rate during late rearing for pullets fed on non-daily feeding schedules is attributed to worse feed conversion (Zuidhof et al., 2015). Additionally, the 4/3 treatment had a lower proportion of mature pullets than the control treatment at the end of rearing. While average body weights in our research met the breeder's performance objective (Aviagen, 2011), there was poor

body weight uniformity for all treatments at the end of rearing. Certainly, the pullets on the 4/3 schedule had the lowest body weight uniformity (>14%) and were lighter than control pullets at the end of rearing. The lower body weight and the poorer body weight uniformity in the pullets on the 4/3 schedule can explain the lower percentage of mature pullets compared to pullets on the control treatment. Pullets on the graduated treatment displayed a compensatory growth rate when the feeding frequency switched from non-daily to daily, and the body weight and the percentage of mature pullets were similar between the graduated and the control treatment at the end of rearing. For this reason, moving forward the transition from non-daily feeding schedules to daily feeding (a few weeks prior to photostimulation) can keep body weights on target at a mature condition at the end of rearing.

Our results indicate the body weight uniformity remained similar in pullets fed the alternative diet and on the 4/3 schedule during mid rearing. However, body weight uniformity decreased during this same period for pullets on the control and on the graduated schedule when treatment switched from the 4/3 to the 5/2 schedule. The relationship among alternative diets, feeding motivation, and body weight uniformity is not straightforward. Qualitative restriction is expected to have a better body weight uniformity than quantitative feed restriction due to larger daily feed allotment, but scientific data do not support this hypothesis (Sandilands et al., 2005, 2006; Morrissey et al., 2014b; Zuidhof et al., 2015). The effect of alternative diets on body weight uniformity might differ based on dietary ingredients, extra feed allotment, and feed restriction level (Bennett and Leeson, 1989; Savory et al., 1996). In our experiment, body weight uniformity was better during mid rearing for pullets on the feeding strategies that increased feed allotment above 50% extra daily feed allotment: the alternative and the 4/3 schedule compared to control. In the case of non-daily feeding, Zuidhof et al. (2015) reported a better body weight uniformity on skip-a-day feeding compared to daily feeding at the end of rearing, although we and others (de Beer and Coon, 2007) found no differences between daily and non-daily feeding on body weight uniformity. Nevertheless, the body weight uniformity of pullets under the graduated treatment improved during the compensatory growth period when these pullets moved from non-daily to daily feeding at the end of rearing. Results from the feeding motivation test suggest that the feeding rate increased with age, unaffected by treatment. Therefore, broiler breeders might have learned to eat as quickly as possible under competitive feeding conditions as may occur with commercial stocking density and limited feeder space. Sandilands et al. (2006) suggested that individual variation in response to dietary diluents and appetite suppressants can explain the lack of difference in body weight uniformity. Therefore, higher feed allotments can maintain body weight uniformity during mid rearing, when feed restriction level was the highest.

Pullets fed on the alternative diet had an increasing litter moisture during early rearing compared to those on the control diet, which coincided with an increased presence of foot lesions during the same period. Similar results were reported in Taira et al. (2014) showing that the average score for footpad dermatitis increased in broilers above 40% litter moisture. Wet litter in broiler breeder flocks can result from water spillage due to drinker pecking (Savory and Maros, 1993; Hocking et al., 2001; Sandilands et al., 2005) and overdrinking (Savory et al., 1992). Sandilands et al. (2005) reported excessive litter moisture in the control treatment due to drinker pecking, while the alternative diet was associated with a higher drinking behavior than control. Although the authors did not observe a negative impact on litter quality, their stocking density was lower than the one used in the current experiment. Others reported higher relative water content of excreta in alternative diets at 40% sugar beet pulp (soluble dietary fiber) but not at 60% oat hulls (insoluble dietary fiber; Savory et al., 1996) compared to a control diet. Under our simulated commercial conditions, the alternative diet led to higher litter moisture probably due to heavier, and probably wetter, excreta production. In summary, the 3 alternative feeding strategies had beneficial effects on feather coverage, feeding motivation during early rearing, and some indicators of stress response (lower number of fault bars in feathers and slower increase of B/L ratio), compared to the control treatment. The 4/3 schedule had a slower growth rate at the end rearing that contributed to a lower percentage of mature pullets, but management practices that improve uniformity can overcome this effect. Pullets on the graduated schedule displayed a compensatory growth rate when the feeding frequency was switched from non-daily to daily, and this improved their feed efficiency. Overall, rationed qualitative and non-daily quantitative feed restriction provide benefits for the welfare of broiler breeders compared to daily quantitative feed restriction although each alternative feeding strategy would require specific management practices to promote the welfare of broiler breeder pullets.

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