Timing of growth affected broiler breeder feeding motivation and reproductive traits

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ABSTRACT The amount and timing of growth are important factors that affect age at first egg, body conformation, reproductive performance, and hunger in broiler breeders. To investigate the effect of growth pattern on feeding motivation and reproductive performance, 10 unique growth trajectories were designed with 2 levels of the amount of early growth and 5 levels of timing of growth around puberty. A 3-phase Gompertz model that described growth in phase 1 (prepubertal), phase 2 (pubertal), and phase 3 (postpubertal) was used to design the growth trajectories. Second growth phase inflection point ($I_2$) was advanced by 0, 5, 10, 15, or 20% of the coefficient estimated from the breeder-recommended target BW. The growth trajectories were designed with 2 discrete levels of total gain in the prepubertal phase ($g_1$); $g_1$ was either the prepubertal phase gain coefficient, estimated from the breeder-recommended BW (Standard $g_1$) target, or 10% higher (High $g_1$). Forty females were randomly assigned to the growth trajectories using a precision feeding (PF) system. Analysis of covariance was conducted on dependent variables in ten 4-wk periods with $g_1$ and periods as discrete fixed effects, $I_2$ as a continuous fixed effect, and age as a random effect. Differences were reported at $P \leq 0.05$. For every week of earlier $I_2$, body weight at photostimulation (BWPS) increased by 126 g; BW at first egg (BWFE) increased by 94 g; 24 wk shank length increased by 0.038 and 1.495 mm in the Standard $g_1$ and High $g_1$ treatments; 24 wk body fat increased by 0.38%; pullets came to lay earlier by 0.49 d; egg weight (EW) increased by 0.27 g; egg production and egg mass (EM) increased by 0.33 egg/hen/d and 0.916 g/d in the High $g_1$ treatment but decreased by 0.27 egg/hen/d and 0.29 g/d in the Standard $g_1$ treatment, respectively. Increasing $g_1$ reduced feeding motivation index by 1.6 and 0.8 visits/meal during rearing and laying phase, respectively. Earlier pubertal growth showed prominent effects on the reproductive performance.

Key words: broiler breeder, feed restriction, Gompertz model, hunger

INTRODUCTION

Broiler breeders are subjected to feed restriction programs to control excessive growth. In contrast with increasing growth rate in broilers (Zuidhof et al., 2014), broiler breeder BW targets have changed very little over the past decades (Renema et al., 2007). Thus, the gap between growth potential of broilers and broiler breeder target BW is increasing, which has resulted in increased feed restriction intensity. Reducing feed consumption to the levels required to control BW has created welfare concerns in underfed breeders (van Krimpen and de Jong, 2014). Some modern broiler breeder pullets do not have sufficient fat reserves to undergo sexual maturation due to severe feed restriction (van Emous et al., 2015; van der Klein et al., 2018a, b; Zuidhof, 2018). Leading up to the onset of lay, breeders should have adequate fleshing (body condition) with optimum levels of protein mass and fat tissue available. There is evidence to suggest that a minimum amount of body fat may be required for broiler breeder pullets to reach sexual maturity (Bornstein et al., 1984; Sun et al., 2006). Kwakkel et al. (1993) described the growth of the body and chemical components of laying hens in a multiphasic manner. They reported that after 11 wk of age, protein and fat deposition was mainly related to the development of the reproductive tract and abdominal fat deposition, respectively. In layers, skeletal frame size can be indirectly assessed by measuring shank length (Kwakkel et al., 1998). Robinson et al. (2007) noted that feed restriction can also limit broiler breeder shank length throughout the rearing period.

Reproductive performance has been compromised by both unrestricted BW in female breeders
(Robinson et al., 1993; Heck et al., 2004) and severe feed restriction (Wilson and Harms, 1986). However, egg production and egg weight (EW) of unrestricted precision fed breeders did not change in response to a 2,007 g increase in the 22 wk BW compared to the standard BW group (Zukiwsky et al., 2021). In another study, high BW hens produced 1.39 times more eggs/hen than standard BW hens from 32 to 55 wk of age (van der Klein et al., 2018b). All high BW hens commenced egg production by the end of their experiment, whereas 37.6% of standard BW hens under 12L:12D photoschedule did not come to lay. The authors hypothesized that current breeder-recommended BW targets may not allow for sufficient body reserves required for the onset of lay in the standard BW hens. They concluded that increasing BW target provided the high BW hens with sufficient metabolic trigger to commence and sustain egg production.

Potential approaches to reduce the intensity of feed restriction in broiler breeders have been investigated in various studies through diet dilution (Zuidhof et al., 1995; Savory and Lariviere, 2000), relaxed feed restriction (Hocking et al., 2002a; Bruggeman et al., 2005; Zukiwsky et al., 2021), and introduction of alternative genetic stock (Heck et al., 2004; Bruggeman et al., 2005). Hocking et al. (2002a) found that increasing target BW by 20% at 18 wk of age did not affect egg or chick production. They reported no difference in the welfare traits (measure of immune function, physiological indices of stress, and behavioral changes) of the hens, which indicated no real benefit of the relaxed feed restriction protocols tested in their studies (Hocking et al., 2001, 2002b). Zukiwsky et al. (2021) increased broiler breeders target BW gain during prepubertal and pubertal phases incrementally up to 22.5% above the recommended BW target. They included a group of unrestricted birds in their study. Some of the unrestricted pullets commenced egg production 2 wk prior to photostimulation. These results strongly suggest that body composition and metabolic status have a role in triggering sexual maturation. Notably, the authors reported that relaxing growth restriction up to 22.5% above the recommended BW target decreased hunger in hens during laying phase but not in pullets during the rearing phase. Hadinia et al. (2020) increased broiler breeder’s dietary energy by 302 kcal/kg from 22 to 26 wk of age. The percentage of birds which commenced laying was 100% in the high ME intake treatment and 30% in the low ME intake treatment. They concluded higher ME intake advanced the activation of hypothalamus-pituitary-gonadal axis, stimulated reproductive hormone levels, and increased lipid deposition in the body of high ME intake treatment group.

Designing strategic growth curves for broiler breeders for systematic evaluation was the main interest behind the current study. The objective of the current study was to evaluate the effect of increased BW gain during prepubertal growth phase and earlier pubertal growth phase on hunger, reproductive performance, body frame size, and body fat in broiler breeder pullets and hens.

**MATERIALS AND METHODS**

The animal protocol for the study was approved by the University of Alberta Animal Care and Use Committee for Livestock and followed the Canadian Council on Animal Care Guidelines and Policies (CCAC, 2009).

**Experimental Design**

The current experiment was conducted as a randomized controlled trial. A total of 40 female Ross 708 broiler breeder pullets were equally and randomly assigned to 10 growth trajectories (Figure 1). Growth trajectories

![Figure 1](image-url)
were designed with 2 levels of the amount of early growth and 5 levels of timing of growth around puberty. Coefficients of growth parameters for breeder-recommended growth trajectory were estimated using a 3-phase Gompertz model fit to the breeder-recommended target BW (Aviagen, 2016). The model had the form (Zuidhof, 2020):

$$BW_t = \sum_{i=1}^{3} \frac{g_i e^{x-p-b_i(t-t_i)}}{C_0} + \epsilon_t$$

where $BW_t$ was BW (kg) at time $t$ (wk); $g_i$ was the total amount of gain (kg) accruing in phase $i$; $b_i$ was the growth rate coefficient for the $i^{\text{th}}$; $t$ was age (wk); $t_i$ was the inflection point (wk), or the age at which growth for phase $i$ reached its maximum rate; and $\epsilon_t$ was the residual error with an expected value of 0, and a normally distributed variance estimated by the software $\epsilon_t \sim N(0, SD^2)$; $i$ was the growth phase ($i = 1$ to 3) where phase 1, 2, and 3 corresponded roughly to prepubertal, pubertal, and postpubertal growth phases, respectively. Other growth trajectories were designed with 2 levels of the prepubertal phase gain coefficient ($g_1$) as discrete variables; $g_1$ was either the estimated gain for phase 1 derived from the breeder-recommended standard BW (Standard $g_1$) target, or 10% higher (High $g_1$). The coefficient $I_2$, which defined the inflection point of the pubertal growth phase ($I_2$), was advanced by 0, 5, 10, 15, or 20% of the coefficient estimated when fitting to the breeder-recommended target BW. $I_2$ was a continuous variable within both the Standard $g_1$ and High $g_1$ groups. The BW trajectories were applied to each individual bird using a precision feeding (PF) system. Therefore, each bird was an experimental unit.

**Animals and Management**

The pullets (n = 40) were housed in a single pen containing 2 PF systems, from hatch to 43 wk of age at a stocking density of 3.0 birds per m$^2$. All birds were fed a commercial diet: starter (crumble; ME 2726 kcal/kg, 21.0% CP, 1.00% Ca, and 0.45% available P) from hatch to d 34; grower (mash; ME 2799 kcal/kg, CP 15.0%, 0.79% Ca, and 0.44% available P) from d 35 to d 179; and breeder diet (crumble; ME 2798 kcal/kg, 15.3% CP, 3.30% Ca, and 0.38% available P) from d 180 onward. Water was provided ad libitum throughout the experiment. The photoschedule was 24L:0D (100 lx) from d 0 to 3 then reduced to 8L:16D (15 lx) on d 4. Pullets were photostimulated at wk 22 by increasing the photoperiod to 11L:13D (20 lx); to 12L:12D (25 lx) on wk 23, then at wk 24 to 13L:11D (50 lx) for the remainder of the experiment. Each PF station had 5 green LED lights (2 lx) that illuminated the inside for 24 h/d so that birds could see their way through the station during the scotophase, without causing photostimulation (Rodriguez, 2017). Room temperature was maintained at 33°C during the first 2 d, and from d 3 onwards temperature was gradually reduced to 20°C by wk 5. A trap nest with 8 nesting sites and a nest box with 8 nesting sites equipped with RFID readers which identified and weighed eggs of individual hens were installed in the room at 14 wk of age; thus, the pullets had the chance to adapt to the nesting system prior to the onset of lay.

All birds were fed individually using a PF system (Zuidhof et al., 2019) that permitted feed intake levels appropriate to achieve the target growth trajectories of each individual bird. Each PF station consisted of 2 motorized entry doors, a sorting and feeding stage, a feeder, and a ramp giving access to the sorting stage. In addition to feed availability from the PF station, supplemental feed was provided on paper plates located around the ramp, on the ramp, and throughout the station and was gradually removed over the first wk to encourage chicks to enter the station individually to reach the feeder. During the training period (first 2 wk), the chicks were placed on the ramp, sorting stage, and feeding stage to get trained to use the PF stations. At 14 d of age each bird was equipped with a wing band containing radiofrequency identification (RFID) transponder to be recognized individually by the PF system. Birds were individually weighed by the PF system in real-time. The treatment BW trajectories were uploaded to the PF system on 14 d of age. The PF system provided access to a meal if the individual birds’ real-time BW was equal or less than the pre-programmed target BW; otherwise, the system gently ejected the birds from the PF station. The chicks were weighed manually daily during the first 3 wk to confirm growth and adoption to the PF system. Feed intake and visit frequency were checked daily to ensure all birds were accessing the PF system. Chicks were provided with additional training to adapt to individual feeding within the feeding station if their BW gain was less than 5 g. FI was less than 2 g, or had less than 3 station visits over the last 24 h. The birds had access to the PF system 24 h per day throughout the experiment.

**Data Collection**

The birds were weighed manually at the same time every morning during the training period. After individual feeding started on d 14, the PF stations recorded individual bird real-time BW and feed intake information upon entry into the station (Zuidhof et al., 2017). The station visit frequency, meal frequency, size of each meal, and ADFI were calculated from the PF system database.

At 24 wk of age, right shank (tibiotarsus) length was measured using digital calipers (Model CD-8°C, Mitutoyo, Japan) from the top of the flexed hock joint to the bottom of the footpad. Simultaneously, abdominal skinfold thickness was measured: each bird was held in standing position with the abdominal skin midway between the vent and the posterior end of the keel bone (sternum) grasped firmly between the tip of the thumb and forefinger of the nondominant hand then lifted such that the skin and subcutaneous fat were drawn away from the underlying tissues. A skinfold caliper (Model Harpenden C-136) was then placed perpendicular to the skin fold, dial up, approximately 1-cm away from the

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finger and thumb. While maintaining the grasp of the skinfold, the Caliper was gently released so that full tension was placed on the skinfold. The dial was read to the nearest 0.50 mm, 1 to 2 s after the spring tension had been fully applied. Body fat as a percentage of BW was estimated using the following model.

\[
\text{Body fat} \, (\%) = 24.83 + 6.75 \times (\ln \text{skinfold}) - 3.87 \times \text{BW},
\]

where skinfold was abdominal skinfold thickness (cm), and BW was measured in kg. The model was created using data from Ross, Avian, and Sex-Links strains with an \( R^2 = 0.63 \) (Latshaw and Bishop, 2001).

The cloaca of all hens was palpated daily in the morning just after initiation of photoperiod to detect hard-shelled eggs in the shell gland. Presence or absence of a hard-shelled egg in the shell gland was recorded daily for each hen. The palpation records were used to determine age at first egg (AFE) and daily oviposition records of individual birds from 20 to 43 wk. Eggs were collected from nest boxes, weighed, and assigned to individual birds daily. Over the duration of the study, there were a total of 10 floor eggs. Floor eggs were assigned to the hen that laid the egg according to palpation records that were cross-referenced with daily records of hens that had laid an egg in the nest boxes. Body weight was evaluated in 2 wk periods from 3 to 42 wk of age. Average daily feed intake and feed seeking behavior (daily station visit:meal ratio) were evaluated from 3 to 42 wk of age. Average daily feed intake and feed seeking behavior (daily station visit:meal ratio) were evaluated in 4-wk periods for the rearing (3–6, 7–10, 11–14, 15–18, and 19–22 wk of age) and laying (23–26, 27–30, 31–34, 35–38, and 39–42 wk of age) phases, separately. Egg production, EW, and egg mass (EM) were evaluated in these same laying phase time periods.

**Statistical Analysis**

Analysis of covariance was conducted on hen-day egg production, EW, EM, station visit frequency, meal frequency, meal size, and visit:meal ratio variables using the MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC), with \( g_1 \) and time period as discrete sources of variation, and \( I_2 \) as a continuous predictor variable. Period was included in the model as a random effect with individual bird as the subject to account for within-bird variation. The same analysis was conducted on shank length, estimated body fat, AFE, BW at photostimulation (BWPS), and BW at first egg (BWFE) without including period in the analysis. Pairwise differences between means within each period were determined using Tukey’s HSD test and were reported as different when \( P \leq 0.05 \). Trends were reported where 0.05 < \( P \leq 0.10 \).

**RESULTS AND DISCUSSION**

Standard coefficients of growth parameters in the 3-phasic Gompertz model were estimated for Ross 708 breeder-recommended BW trajectory (Table 1; Figure 1). Then \( g_1 \) was increased by 10% to create High \( g_1 \) BW trajectories (Table 1). The breeder-recommended \( I_2 \) at 22.29 wk of age predicted accumulation of 90% of the total growth for the pubertal phase in approximately 20 wk, from 17 to 37 wk of age. Pubertal inflection point was advanced in both Standard and High \( g_1 \) treatments creating inflection points that varied by 1.1 wk (7.8 d) in the range of 17.82 to 22.29 wk of age. Correspondingly, the predicted timeframe for accumulation of 90% of the total pubertal growth advanced by 7.8 d with each 5% advancement of \( I_2 \) (Table 1; Figure 2).

**Body Weight**

Body weight was similar across BW trajectories from 3 to 6 wk of age (Table 2). Target BW might have not diverged enough among BW trajectories (Figure 1) to

| Growth parameter | Standard \( g_1 \) | High \( g_1 \) |
|------------------|------------------|--------------|
|                  | \( I_2-0\% \) | \( I_2-5\% \) | \( I_2-10\% \) | \( I_2-15\% \) | \( I_2-20\% \) | \( I_2-0\% \) | \( I_2-5\% \) | \( I_2-10\% \) | \( I_2-15\% \) | \( I_2-20\% \) |
| \( n^3 \)        | 4                | 4             | 4             | 4             | 4             | 4             | 0             | 0             | 0             | 1             | 0             |
| Mortality        | 0                | 0             | 0             | 0             | 0             | 0             | 0             | 0             | 0             | 0             | 0             |
| \( g_1 \) (kg)   | 1.880            | 1.880         | 1.880         | 1.880         | 1.880         | 2.068         | 2.068         | 2.068         | 2.068         | 2.068         | 2.068         |
| \( b_1 \) (wk)   | 0.147            | 0.147         | 0.147         | 0.147         | 0.147         | 0.147         | 0.147         | 0.147         | 0.147         | 0.147         | 0.147         |
| \( I_1 \) (wk)   | 7.30             | 7.30          | 7.30          | 7.30          | 7.30          | 7.30          | 7.30          | 7.30          | 7.30          | 7.30          | 7.30          |
| \( g_2 \) (kg)   | 1.696            | 1.696         | 1.696         | 1.696         | 1.696         | 1.696         | 1.696         | 1.696         | 1.696         | 1.696         | 1.696         |
| \( b_2 \) (wk)   | 0.208            | 0.208         | 0.208         | 0.208         | 0.208         | 0.208         | 0.208         | 0.208         | 0.208         | 0.208         | 0.208         |
| \( I_2 \) (wk)   | 22.29            | 22.16         | 20.05         | 18.94         | 17.92         | 22.29         | 22.16         | 20.05         | 18.94         | 17.92         | 18.94         |
| \( b_3 \) (kg)   | 0.451            | 0.451         | 0.451         | 0.451         | 0.451         | 0.451         | 0.451         | 0.451         | 0.451         | 0.451         | 0.451         |
| \( I_3 \) (wk)   | 54.85            | 54.85         | 54.85         | 54.85         | 54.85         | 54.85         | 54.85         | 54.85         | 54.85         | 54.85         | 54.85         |

1The coefficients for “Standard \( g_1 \), Standard \( I_2-0\% \)” BW trajectory were estimated by fitting a 3-phase Gompertz model to the breeder-recommended Ross 708 female broiler target BW. General model form was \( BW_i = \sum_{i=1}^{\infty} e^{g_i \times \text{exp}(\text{exp}(-t_i \times \text{time}))} \) where \( BW_i \) was BW (kg) at time \( t_i \); \( g_i \) was the total amount of gain (kg) accruing in phase \( i \); \( b_3 \) was the growth rate coefficient; \( t_i \) was age (wk); \( I_i \) was the inflection point (wk), or the age at which growth for phase \( i \) reached its maximum rate.

2\( g_1 \) was either the gain coefficient for the prepubertal phase, estimated from the breeder-recommended standard BW gain (Standard \( g_1 \), target, or 10% higher (High \( g_1 \)). Second growth phase (pubertal) inflection point (\( I_2 \)) was advanced such that \( I_2-0\% = 22.29 \) wk; \( I_2-5\% = 21.16 \) wk; \( I_2-10\% = 20.05 \) wk; \( I_2-15\% = 18.94 \) wk; \( I_2-20\% = 17.82 \) wk.

3\( n \) was number of birds grown in each growth trajectory.
detect significant differences in bird BW by 6 wk of age. High g1 pullets had a greater average BW than that of their Standard g1 counterparts from 7 to 8 wk of age. However, earlier I2 did not increase BW within the Standard g1 and High g1 treatments by 8 wk of age. Pullet BW started to diverge within the Standard g1 and High g1 treatments starting at 9 wk of age due to earlier I2 (earlier pubertal growth). Increasing g1 by 10% increased BWPS for High g1 hens by 6.4% (167 g) compared to that of the Standard g1 hens (P < 0.001, Table 3). For every week earlier I2, BWPS and BWFE increased by 126 g (P < 0.001) and 94 g (P < 0.001). After 36 wk of age, there were no differences among bird BW within the Standard g1 and High g1 treatments (Table 2) as the target growth trajectories started to converge (Figure 1).

Shank Length and Body Fat

Shank length and estimated body fat were used as proxies for body frame size and body composition, respectively. Advancing the inflection point of the second (pubertal) growth phase increased shank length at 24 wk of age by 0.038 and 1.495 mm/wk within the Standard g1 and High g1 treatments, respectively (P = 0.046, Table 4). Renema et al. (2007) noted that feed restriction can limit shank length throughout the rearing phase. Achieving adequate body frame development threshold provides the bird the foundation for a successful laying cycle (Shi et al., 2020). Increasing g1 by 10% did not affect the estimated body fat. For every week of earlier pubertal growth, estimated body fat increased by 0.38% (P = 0.013, Table 4). It was shown that carcass fat at sexual maturity is between 11 and 15% of total BW (Joseph et al., 2000; Renema et al., 2007), which is not consistent with the estimated body fat in the current study (8.0 ± 0.4 and 8.5 ± 0.4% for the Standard g1 and High g1 treatments, respectively). This might be due to lower body fat in Ross 708 strain (Renema et al., 2007) and the fact that body fat has decreased in modern broiler breeders (Caldas et al., 2018). To commence egg production and support adequate reproductive performance in broiler breeders, a minimum percentage of body fat is required (Sun and Coon, 2009; van Emous et al., 2013). In the current study all birds reached the sexual maturity and commenced egg laying; thus, the minimum body fat threshold is likely below 8%.

Age at First Egg

Standard g1 and High g1 hens commenced lay at almost the same age (176 d, Table 3). Age at first egg advanced by 0.49 d/wk of earlier I2 (P = 0.046, Table 3). This might be because birds with earlier pubertal growth had higher estimated body fat, as a measure of body composition, and longer shank length, as a measure of body frame size, compared to their counterparts with standard I2 (Table 4). These birds may have reached the BW and body composition thresholds required for onset of lay because of earlier pubertal growth. Thus, achieving those thresholds may have provided sufficient metabolic triggers for sexual maturation. Extra ME and nutrients at this time can advance the sexual maturation process in broiler breeder individuals by advancing the activation of the hypothalamus-pituitary-gonadal axis and increasing body lipid deposition (Renema et al., 1999; Hadinia et al., 2020). However, Renema et al. (2007) did not find advancement in AFE when they increased 12-wk target BW by 150 and 200% and photostimulated the birds at 22 wk of age. We previously reported that there is individual variation in the thresholds for sexual maturity because each bird might have a unique BW threshold to reach sexual maturity (Zukiwsky et al., 2021).

Egg Production, EM, and EW

Compared to the Standard g1 hens High g1 hens produced one more egg/hen/period (P = 0.013, Table 5) and 2.95 g/d greater EM (P = 0.022). High and Standard g1 hens produced 110 and 105 eggs/hen throughout the laying phase, respectively (P = 0.047; data not shown). Increasing BW by 20% (430 g) at 20 wk of age increased number of eggs per hen housed (Ekmay et al., 2012). In the current study for every week of earlier I2, BW at 20 wk of age increased by 6.5%; number of eggs/hen increased by 0.33 egg/hen for the High g1 treatment and decreased by 0.27 egg/hen and for the Standard g1
Table 2. Effect of BW trajectory\(^1\) (W) and time period on BW during rearing and laying phases in Ross 708 broiler breeder.

| Phase | Period | Standard \(g_1\) | High \(g_1\) |
|-------|--------|----------------|-------------|
|       |        | L\(_2\)-0% | L\(_2\)-5% | L\(_2\)-10% | L\(_2\)-15% | L\(_2\)-20% | L\(_2\)-0% | L\(_2\)-5% | L\(_2\)-10% | L\(_2\)-15% | L\(_2\)-20% |
|       |        | LSMean  | SEM  | LSMean  | SEM  | LSMean  | SEM  | LSMean  | SEM  | LSMean  | SEM  | LSMean  | SEM  |
| Rearing | 3     | 341     | 22.9 | 332     | 22.9 | 302     | 26.5 | 320     | 26.5 | 328     | 22.9 | 360     | 22.9 |
|        | 5     | 761\(^b\) | 6.5  | 760\(^b\) | 6.5  | 760\(^b\) | 7.5  | 760\(^b\) | 6.5  | 836\(^a\) | 6.5  | 834\(^a\) | 7.5  |
|        | 7     | 957\(^a\) | 1.0  | 957\(^a\) | 1.0  | 967\(^a\) | 1.2  | 967\(^a\) | 1.0  | 1,051\(^b\) | 1.0  | 1,052\(^b\) | 1.2  |
|        | 9     | 1,306\(^b\) | 1.0  | 1,306\(^b\) | 1.0  | 1,315\(^b\) | 1.2  | 1,315\(^b\) | 1.0  | 1,425\(^c\) | 1.0  | 1,438\(^c\) | 2.2  |
|        | 11    | 1,462\(^c\) | 1.3  | 1,513\(^c\) | 1.3  | 1,587\(^c\) | 1.6  | 1,611\(^c\) | 1.3  | 1,604\(^c\) | 1.3  | 1,652\(^c\) | 1.6  |
|        | 13    | 1,495\(^d\) | 0.9  | 1,771\(^d\) | 0.9  | 1,888\(^d\) | 1.1  | 2,166\(^d\) | 1.1  | 2,674\(^d\) | 0.9  | 2,836\(^d\) | 1.1  |
|        | 15    | 1,573\(^e\) | 1.1  | 1,778\(^e\) | 1.1  | 2,078\(^e\) | 1.3  | 2,237\(^e\) | 1.3  | 2,753\(^e\) | 1.1  | 2,975\(^e\) | 1.3  |
|        | 17    | 1,683\(^f\) | 1.1  | 1,991\(^f\) | 1.1  | 2,327\(^f\) | 1.3  | 2,718\(^f\) | 1.3  | 3,260\(^f\) | 1.1  | 3,531\(^f\) | 1.3  |
|        | 19    | 1,826\(^g\) | 1.1  | 2,221\(^g\) | 1.1  | 2,362\(^g\) | 1.3  | 2,999\(^g\) | 1.3  | 3,578\(^g\) | 1.1  | 3,974\(^g\) | 1.3  |
|        | 21    | 2,080\(^h\) | 1.1  | 2,438\(^h\) | 1.1  | 2,665\(^h\) | 1.3  | 3,196\(^h\) | 1.3  | 3,758\(^h\) | 1.1  | 4,240\(^h\) | 1.3  |
| Laying | 23    | 2,556\(^j\) | 2.8  | 3,006\(^j\) | 2.8  | 3,256\(^j\) | 3.3  | 3,820\(^j\) | 3.3  | 4,520\(^j\) | 2.8  | 5,280\(^j\) | 3.3  |
|        | 25    | 2,813\(^i\) | 16.2 | 3,292\(^i\) | 16.2 | 3,756\(^i\) | 18.7 | 4,316\(^i\) | 18.7 | 5,020\(^i\) | 16.2 | 5,820\(^i\) | 16.2 |
|        | 27    | 3,177\(^j\) | 16.7 | 3,522\(^j\) | 16.7 | 4,034\(^j\) | 17.3 | 4,646\(^j\) | 17.3 | 5,366\(^j\) | 16.7 | 6,156\(^j\) | 16.7 |
|        | 31    | 3,863\(^k\) | 13.9 | 3,325\(^k\) | 13.9 | 3,595\(^k\) | 16.1 | 3,646\(^k\) | 16.1 | 3,758\(^k\) | 13.9 | 4,000\(^k\) | 13.9 |
|        | 33    | 3,402\(^l\) | 13.5 | 3,402\(^l\) | 13.5 | 3,446\(^l\) | 15.6 | 3,466\(^l\) | 15.6 | 3,578\(^l\) | 13.9 | 3,996\(^l\) | 13.9 |
|        | 35    | 3,471\(^m\) | 18.5 | 3,471\(^m\) | 18.5 | 3,483\(^m\) | 21.4 | 3,483\(^m\) | 21.4 | 3,578\(^m\) | 18.5 | 4,000\(^m\) | 18.5 |
|        | 37    | 3,478\(^n\) | 17.8 | 3,480\(^n\) | 17.8 | 3,505\(^n\) | 20.5 | 3,497\(^n\) | 20.5 | 3,646\(^n\) | 17.8 | 4,000\(^n\) | 17.8 |
|        | 39    | 3,501\(^o\) | 22.0 | 3,506\(^o\) | 22.0 | 3,528\(^o\) | 25.4 | 3,556\(^o\) | 25.4 | 3,711\(^o\) | 22.0 | 4,000\(^o\) | 22.0 |
|        | 41    | 3,530\(^p\) | 25.0 | 3,532\(^p\) | 25.0 | 3,548\(^p\) | 28.8 | 3,558\(^p\) | 28.8 | 3,732\(^p\) | 25.0 | 4,000\(^p\) | 25.0 |

1A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder-recommended target BW to estimate the model coefficients. BW trajectories were designed with two levels of prepubertal BW gain (\(g_1\)) coefficient and 5 levels of pubertal growth phase inflection point (I\(_2\)) coefficient. \(g_1\) was estimated from the breeder-recommended standard BW gain (Standard \(g_1\)) target, or 10% higher (High \(g_1\)). Second growth phase (pubertal) inflection point (I\(_2\)) was advanced such that I\(_2\)-0% = 22.29 wk, I\(_2\)-5% = 21.16 wk, I\(_2\)-10% = 20.05 wk, I\(_2\)-15% = 18.94 wk, I\(_2\)-20% = 17.82 wk.

\(^{a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p}\)Means within rows with no common superscript differ (\(P < 0.05\)).
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Table 3. Effects of prepubertal BW gain and pubertal growth inflection on BW at photostimulation (BWPS) and BW at first egg (BWFE) of Ross 708 broiler breeder pullets.

| Effect† | g1 | AFE | SEM | BWPS | SEM | BWFE | SEM |
|---------|----|-----|-----|------|-----|------|-----|
| g1      | Standard g1 | 175.7 | 1.3 | 2.614<sup>b</sup> | 2.42 | 2.943<sup>b</sup> | 21.96 |
|         | High g1     | 175.6 | 1.4 | 2.781<sup>a</sup> | 2.64 | 3.112<sup>a</sup> | 23.94 |
| I<sub>2</sub> | 0.49 | 0.83 | 126 | 1.52 | 94  | 13.75 |
| I<sub>2</sub> × g1 | Standard g1 | 0.49 | 0.83 | 126 | 1.52 | 94  | 13.75 |
|         | High g1     | 1.48 | 1.19 | 190 | 2.17 | 59  | 19.71 |

Source of variation

| g1 | P-value | I<sub>2</sub> | P-value | I<sub>2</sub> × g1 | P-value |
|----|---------|--------------|---------|-------------------|---------|
| 0.22 | 0.001 | 0.046 | <0.001 | 0.22 | 0.33 |

†g1: Prepubertal phase gain coefficient estimated by a 3-phasic Gompertz growth model fitted to the standard Ross 708 recommended BW gain target (Standard g1) or 10% higher (High g1). Second growth phase (pubertal) inflection point (I<sub>2</sub>) was advanced such that I<sub>2</sub>-0% = 22.29 wk, I<sub>2</sub>-5% = 21.16 wk, I<sub>2</sub>-10% = 20.05 wk, I<sub>2</sub>-15% = 18.94 wk, I<sub>2</sub>-20% = 17.82 wk.

Table 4. Effects of prepubertal BW gain and pubertal growth inflection on shank length and estimated body fat content at 24 weeks of age in Ross 708 broiler breeder hens.

| Effect† | g1 | Shank length<sup>2</sup> | SEM | Body fat<sup>3</sup> | SEM |
|---------|----|----------------|-----|----------------|-----|
| g1      | Standard g1 | 98.4 | 0.8 | 8.04 | 0.38 |
|         | High g1     | 99.9 | 0.8 | 8.47 | 0.38 |
| I<sub>2</sub> | -0.038 | 0.511 | -0.38 | 0.24 |
| I<sub>2</sub> × g1 | Standard g1 | -0.038 | 0.511 | -0.38 | 0.24 |
|         | High g1     | -1.495 | 1.216 | -0.53 | 0.59 |

Source of variation

| g1 | P-value | I<sub>2</sub> | P-value | I<sub>2</sub> × g1 | P-value |
|----|---------|--------------|---------|-------------------|---------|
| 0.19 | 0.44 | 0.036 | 0.013 | 0.046 | 0.07 |

†g1: Prepubertal phase gain coefficient estimated by a 3-phasic Gompertz growth model fitted to the standard Ross 708 recommended BW gain target (Standard g1) or 10% higher (High g1). Second growth phase (pubertal) inflection point (I<sub>2</sub>) was advanced such that I<sub>2</sub>-0% = 22.29 wk, I<sub>2</sub>-5% = 21.16 wk, I<sub>2</sub>-10% = 20.05 wk, I<sub>2</sub>-15% = 18.94 wk, I<sub>2</sub>-20% = 17.82 wk.

‡Shank length = Tibiotarsus measured from top of flexed hock joint to bottom of footpad.

³Body fat (%) estimated by Body fat (%) = 24.83 + 6.75 (ln skinfold) − 3.87 BW where skinfold is abdominal skinfold thickness in cm and BW is in kg (Latshaw and Bishop, 2001).

Feeding Motivation

The frequency of daily station visits, visit:meal ratio, and meal size could all be indicators of feeding motivation. During the rearing phase, Standard g1 pullets had approximately 7 more daily station visits compared to the High g1 pullets (P = 0.005, Table 6), which would be
consistent with a higher degree of hunger in the Standard g1 birds. For every week that I2 was advanced, the station visit frequency decreased by 2.55 visits in the Standard g1 pullets and increased by 1.08 visits in the High g1 group. Birds with earlier I2 started to accumulate pubertal gain earlier than those with standard I2 resulting in a lower degree of feed restriction. Thus, it is possible that those Standard g1 birds with earlier I2 were less hungry and less motivated to enter the feeding station to seek feed compared to their counterparts with standard I2. High g1 pullets might have approached a point of satiety because of having 10% higher g1; thus, earlier I2 did not decrease their daily station visits.

During the laying phase, the frequency of daily station visits was not affected by g1 but was increased by 0.83 and 4.97 visits/d/wk of earlier I2 (P = 0.002).

Increasing g1 by 10% increased meal frequency during rearing (P < 0.001, Table 6) and laying phase (P = 0.041, Table 7) because of increased target BW in the High g1 birds to support maintenance requirements, prepubertal growth (muscle and skeletal development) during rearing, pubertal growth (development of reproductive tract and fat deposition) toward the end of rearing, and egg production throughout the laying phase. Meal frequency increased by 0.34 meal/wk of earlier I2 during the rearing phase (P < 0.001, Table 6). This was

| Table 5. Effects of prepubertal BW gain and pubertal growth inflection on egg weight (EW), egg mass (EM), and number of eggs during 4 wk periods from 23 to 42 wk of age of Ross 708 broiler breeder hens. |
|---|---|---|---|---|---|---|---|
| Effect\(^1\) | g1 | Period (wk) | EW | SEM | EM | SEM | Egg | SEM |
|---|---|---|---|---|---|---|---|
| g1 | Standard g1 | 23 to 26 | 59.2 | 0.4 | 42.73\(^a\) | 0.70 | 20\(^a\) | 0.3 |
| | High g1 | 60.0 | 0.4 | 45.68\(^a\) | 0.76 | 21\(^a\) | 0.3 |
| | | 27 to 30 | 52.8\(^b\) | 0.5 | 20.15\(^c\) | 1.70 | 11\(^d\) | 0.9 |
| | | 31 to 34 | 59.6\(^b\) | 0.5 | 44.29\(^b\) | 1.06 | 21\(^a\) | 0.5 |
| | | 35 to 38 | 64.5\(^b\) | 1.1 | 53.73\(^a\) | 1.40 | 24\(^b\) | 0.6 |
| | | 39 to 42 | 64.1\(^i\) | 0.8 | 50.78\(^a\) | 0.90 | 23\(^b\) | 0.4 |
| I2 | Standard g1 | 0.37 | 0.022 | 0.013 | | | |
| | High g1 | 2.55 | 1.15 | | | | |
| Source of variation | g1 | 0.13 | 0.022 | 0.013 | | | |
| | I2 | 0.036 | 0.29 | 0.13 | | | |
| | I2 x g1 | 0.75 | 0.040 | 0.021 | | | |
| | Period | < 0.001 | < 0.001 | < 0.001 | | | |

\(^1\)g1: Prepubertal phase gain coefficient estimated by a 3-phasic Gompertz growth model fitted to the standard Ross 708 recommended BW gain target (Standard g1) or 10% higher (High g1). Second growth phase (pubertal) inflection point (I2) was advanced such that I2-0% = 22.29 wk, I2-5% = 21.16 wk, I2-10% = 20.05 wk, I2-15% = 18.94 wk, I2-20% = 17.82 wk. 

\(^a-d\)Means within columns with no common superscript differ (P < 0.05).

| Table 6. Effects of prepubertal BW gain and pubertal growth inflection on the station visit frequency, meal frequency, feeding motivation index, and meals size during rearing phase of Ross 708 broiler breeder pullets. |
|---|---|---|---|---|---|
| Effect\(^1\) | g1 | Period | Visits | SEM | Meals | SEM | Feeding motivation index\(^2\) | SEM | Meal size | SEM | ADFI | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g1 | Standard g1 | 3 to 6 | 53.7\(^a\) | 1.9 | 7.1\(^a\) | 0.1 | 8.6\(^a\) | 0.3 | 9.0 | 0.1 | 62.9\(^a\) | 0.8 |
| | High g1 | 46.0\(^b\) | 2.1 | 7.6\(^a\) | 0.1 | 7.0\(^b\) | 0.4 | 9.2 | 0.1 | 67.3\(^a\) | 0.9 |
| Period | 7 to 10 | 34.9\(^b\) | 3.2 | 8.4\(^a\) | 0.3 | 6.1\(^a\) | 0.7 | 7.0\(^d\) | 0.3 | 50.6\(^a\) | 2.8 |
| | 11 to 14 | 63.1\(^a\) | 4.0 | 6.2\(^b\) | 0.2 | 11.0\(^e\) | 0.7 | 8.6 | 0.2 | 53.3\(^a\) | 2.0 |
| | 15 to 18 | 55.7\(^b\) | 3.6 | 6.6\(^c\) | 0.1 | 8.9\(^d\) | 0.6 | 8.7 | 0.1 | 56.2\(^a\) | 0.5 |
| | 19 to 22 | 49.5\(^c\) | 2.6 | 7.2\(^c\) | 0.1 | 7.3\(^c\) | 0.5 | 10.3 | 0.1 | 74.0\(^a\) | 1.0 |
| I2 | Standard g1 | 0.75 | 0.040 | 0.021 | | | |
| | High g1 | 0.036 | 0.29 | 0.13 | | | |
| Source of variation | g1 | 0.75 | 0.040 | 0.021 | | | |
| | I2 | < 0.001 | < 0.001 | < 0.001 | | | |

\(^1\)g1: Prepubertal phase gain coefficient estimated by a 3-phasic Gompertz growth model fitted to the standard Ross 708 recommended BW gain target (Standard g1) or 10% higher (High g1). Second growth phase (pubertal) inflection point (I2) was advanced such that I2-0% = 22.29 wk, I2-5% = 21.16 wk, I2-10% = 20.05 wk, I2-15% = 18.94 wk, I2-20% = 17.82 wk. 

\(^2\)Feeding motivation index was defined as daily station visit:meal ratio.

\(^a-d\)Means within columns with no common superscript differ (P < 0.05).
Table 7. Effects of prepubertal BW gain and pubertal growth inflection on the station visit frequency, meal frequency, motivation index, and meals size during laying phase of Ross 708 broiler breeder hens.

| Effect\(^1\) | \(g_1\) | Period | Visits | SEM | Meals | SEM | Feeding motivation index\(^2\) | SEM | Meal size | SEM | ADFI | SEM |
|----------------|----------------|--------|--------|-----|--------|-----|--------------------------|-----|-----------|-----|-------|-----|
| \(g_1\) | Standard \(g_1\) | wk | visit | meals | visits/meal | g/visit | g/day |
| 23 to 26 | 37.8 | 1.5 | 9.4 | 0.2 | 4.8 | 0.2 | 15.1 | 0.2 | 132.9 | 0.9 |
| 27 to 30 | 33.6 | 2.2 | 11.6 | 0.4 | 3.2 | 0.3 | 13.8 | 0.4 | 147.6 | 1.2 |
| 31 to 34 | 37.9 | 2.4 | 9.2 | 0.3 | 5.2 | 0.4 | 18.5 | 0.5 | 159.7 | 1.8 |
| 35 to 38 | 31.4 | 2.4 | 8.2 | 0.3 | 4.1 | 0.3 | 17.5 | 0.5 | 138.3 | 1.4 |
| 39 to 42 | 32.5 | 2.1 | 8.9 | 0.3 | 4.2 | 0.3 | 16.2 | 0.5 | 138.1 | 1.0 |
| \(I_2\) | Standard \(g_1\) | | -8.3 | 0.90 | 0.25 | 0.12 | -0.33 | 0.12 | -0.18 | 0.12 | 1.3 | 0.5 |
| \(I_2\) | High \(g_1\) | | -8.3 | 0.90 | 0.25 | 0.12 | -0.33 | 0.12 | -0.18 | 0.12 | 1.3 | 0.5 |
| \(I_2 \times g_1\) | Standard \(g_1\) | | -4.97 | 1.29 | 0.08 | 0.29 | -0.54 | 0.29 | -0.14 | 0.18 | -1.2 | 0.7 |

\(^1\)\(g_1\): Prepubertal phase gain coefficient estimated by a 3-phasic Gompertz growth model fitted to the standard Ross 708 recommended BW gain target (Standard \(g_1\)) or 10% higher (High \(g_1\)). Second growth phase (pubertal) inflection point \((I_2)\) was advanced such that \(I_2-0\% = 22.29\) wk, \(I_2-5\% = 21.16\) wk, \(I_2-10\% = 20.05\) wk, \(I_2-15\% = 18.94\) wk, \(I_2-20\% = 17.82\) wk.

\(^2\)Feeding motivation index was defined as daily station visit:meal ratio.

\(= 0.001\). As the birds with earlier \(I_2\) increased the level of feed restriction as the birds had access to feed based on their BW. This is in line with an increase in ADFI by 3.9 g/d/wk of earlier \(I_2\). However, for every week of earlier \(I_2\), meal frequency tended to decrease by 0.25 meals/d during the laying phase \((P = 0.055, Table 7)\).

Feeding motivation index was defined as the visit:meal ratio indicating the feed seeking motivation, driven by the number of meals allowed. Feeding motivation index for the Standard \(g_1\) and High \(g_1\) birds was 8.6 and 7.0 visits/meal during the rearing and laying phase, respectively. Earlier growth reduced feeding motivation index for both Standard \(g_1\) and High \(g_1\) pullets. However, using daily station visit frequency on its own showed an increase in “feeding motivation” for those High \(g_1\) pullets with earlier \(I_2\) compared with their counterparts with a standard \(I_2\). Therefore, it could be hypothesized that visit:meal ratio might be a better indicator of feeding motivation compared to daily station visit frequency.

Feeding motivation is affected by both external and internal factors. For instance, feeding motivation in broiler breeders is affected by both increased appetite because of genetic selection (internal) and the availability and allocation of feed in the environment (external). Every day a hen produced an egg, BW of the hen was reduced by the weight of the egg, so the hen qualified for additional feed allocation through the PF system, as the PF feed allocation decision was based on BW. During the laying phase, feeding motivation index increased by 0.33 visits/meal/wk of earlier \(I_2\) \((P < 0.001)\). As the birds with earlier \(I_2\) commenced egg production earlier than those with standard \(I_2\) \((P = 0.046, Table 3)\), they qualified for additional feed allocation as an external feeding motivation. It could have motivated the birds with earlier \(I_2\) to seek feed from the PF system leading to an increased visit:meal ratio.

expected, as feed restriction is reportedly most severe from 8 to 16 wk of age when broiler breeders are restricted 25 to 30% of the intake of unrestricted birds (de Jong and Jones, 2006). Thus, increasing BW target by advancing \(I_2\) decreased the level of feed restriction as the birds had access to feed based on their BW. This is in line with an increase in ADFI by 3.9 g/d/wk of earlier \(I_2\). However, for every week of earlier \(I_2\), meal frequency tended to decrease by 0.25 meals/d during the laying phase \((P = 0.055, Table 7)\).

Feeding motivation index was defined as the visit:meal ratio indicating the feed seeking motivation, driven by the number of meals allowed. Feeding motivation index for the Standard \(g_1\) and High \(g_1\) birds was 8.6 and 7.0 visits/meal during the rearing phase \((Table 6)\) and 4.8 and 4.0 visits/meal during the laying phase \((Table 7)\), respectively. Thus, High \(g_1\) birds had 1.6 and 0.8% lower feeding motivation index than that of the Standard \(g_1\) birds during the rearing and laying phase, respectively. Earlier \(I_2\) reduced feeding motivation index during the rearing phase by 0.75 and 0.16 visits/meal in the Standard \(g_1\) and High \(g_1\) pullets, respectively \((P = 0.038, Table 6)\). A lower reduction in feeding motivation index of High \(g_1\) pullets compared to their Standard \(g_1\) counterparts indicates that increasing \(g_1\) by 10% had already decreased their hunger in such a way that earlier \(I_2\) (further release in growth restriction) just had a minor effect on alleviating their hunger. These results are in line with Savory and Lariviére (2000) who investigated broiler breeder feeding motivation using an operant conditioning system during the rearing phase. The birds were receiving feed as a reward after pecking at a disc implemented in the operant system. The authors measured the number of operant responses in 12 min as a proxy of feeding motivation and found a positive relationship between feed motivation and suppression of growth rate. Their study showed that the number of operant responses decreased by 63, 45, 57, and 62 times per each kg increase in BW at 8, 10, 12, and 14 wk of age, respectively. However, the results of the current study during the rearing phase are in contrast with results from Zukiwsky et al. (2021) who did not observe a decrease in feed seeking behavior during the rearing phase as BW increased up to 22.5% above the recommended BW target. In fact, they used daily station visits as an indicator of feed seeking behavior and did not account for the meal frequency by calculating the visit:meal ratio. In the current analysis, the feeding motivation index accounted for the meal frequency. Earlier pubertal growth reduced feeding motivation index for both Standard \(g_1\) and High \(g_1\) pullets. However, using daily station visit frequency on its own showed an increase in “feeding motivation” for those High \(g_1\) pullets with earlier \(I_2\) compared with their counterparts with a standard \(I_2\). Therefore, it could be hypothesized that visit:meal ratio might be a better indicator of feeding motivation compared to daily station visit frequency.

Feeding motivation is affected by both external and internal factors. For instance, feeding motivation in broiler breeders is affected by both increased appetite because of genetic selection (internal) and the availability and allocation of feed in the environment (external). Every day a hen produced an egg, BW of the hen was reduced by the weight of the egg, so the hen qualified for additional feed allocation through the PF system, as the PF feed allocation decision was based on BW. During the laying phase, feeding motivation index increased by 0.33 visits/meal/wk of earlier \(I_2\) \((P < 0.001)\). As the birds with earlier \(I_2\) commenced egg production earlier than those with standard \(I_2\) \((P = 0.046, Table 3)\), they qualified for additional feed allocation as an external feeding motivation. It could have motivated the birds with earlier \(I_2\) to seek feed from the PF system leading to an increased visit:meal ratio.
Meal size might also be an indicator of hunger and feeding motivation. A larger meal size was related to a faster feed intake rate, as birds had 60 s to eat off the feeder before being ejected from the PF system. Meal size increased by age ($P < 0.001$, Tables 6 and 7) but was not affected by the $g_1$ treatment during rearing and laying phases. During the rearing phase meal size increased by 0.08 and 0.03 g/visit/wk of earlier $I_2$ for the Standard $g_1$ and High $g_1$ pullets, respectively ($P = 0.038$, Table 6). This corresponds with an increase in ADFI by 3.9 g/d/wk of earlier $I_2$ to fulfill nutrient requirements associated with weight gain ($P < 0.001$). Furthermore, High $g_1$ pullets had 5.1 g/d greater ADFI than that of Standard $g_1$ pullets ($P < 0.001$), which was because of decreased feed restriction in the High $g_1$ pullets. During the laying phase, meal size tended to increase by 0.18 g/visit/wk of earlier $I_2$ ($P = 0.068$, Table 7). Earlier $I_2$ decreased ADFI by 1.3 g/d/wk for the Standard $g_1$ hens and increased it by 1.2 g/d/wk for the High $g_1$ birds ($P < 0.001$). This might have been due to higher station visit frequency with earlier $I_2$ for High $g_1$ birds (4.97 visit/wk) compared to that of Standard $g_1$ (0.83 visit/wk) hens during the laying phase (Table 7).

To decrease the gap between broiler breeders and their offspring target BW, and mitigate adverse effects of severe feed restriction, the current study was designed focusing on relaxed growth restriction during prepubertal growth phase and earlier pubertal growth phase. To our knowledge, this is the first investigation of the effects of systematic evaluation of BW targets using designed growth trajectories based on earlier pubertal growth phase in broiler breeders. The results of the current study indicated that the strategy of earlier pubertal growth could reduce hunger in broiler breeders during rearing and laying phase. Furthermore, it allowed female breeders to achieve a sufficient foundation and appropriate fat level for sexual maturation, which advanced sexual maturation. Relaxed feed restriction during prepubertal phase and earlier pubertal growth showed prominent effects on egg production, EM, and EW as proxies for reproductive output.

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**DISCLOSURES**

The authors declare that there is no conflict of interest.

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