A model of pre-pubertal broiler breeder estradiol-17β levels predicts advanced sexual maturation for birds with high body weight or short juvenile day-length exposure

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ABSTRACT As broiler breeders face increased reproductive challenges specifically related to overfeeding, a clear understanding of the physiological effects of BW and rearing photoperiod on reproductive development is needed. The objective was to use mathematical models to compare plasma estradiol-17β (**E2**) concentration to characterize the effect of BW and rearing photoperiod on E2 levels. A 2 × 3 factorial arrangement of treatments was used. Hens (n = 180) were fed with a precision feeding system to allocate feed individually to achieve the breeder-recommended BW curve (Standard) or to a BW curve reaching the 21 wk target at 18 wk (High). Hens were on 8L:16D, 10L:14D, or 12L:12D photoschedules during rearing and were photostimulated at 21 wk. Age at first egg (AFE) was recorded. Plasma E2 levels were determined weekly between week 20 and 28. Two modified Gompertz models described E2 level as a function of (a) chronological or (b) physiological (relative to AFE) age. Timing of E2-inflection point was compared between models and treatments. Differences were reported as significant at $P \leq 0.05$. The chronological age model inferred that High BW reduced the duration between the E2-inflection point and AFE, whereas the physiological age model inferred that High BW only reduced the duration between photostimulation and the E2-inflection point. Hens on the Standard BW treatment had a longer period between photostimulation and the E2-inflection point compared to hens on the High-BW treatment (11.03 vs. 1.50 wk, respectively, based on physiological age). Hens on the 12L:12D photoschedule had a longer period between photostimulation and the E2-inflection point compared to hens on the 8L:16D or 10L:14D photoschedule, both in the Standard and High BW (28.91 vs. 1.78 and 2.40 wk, 2.65 vs. 0.93 and 0.94 wk, respectively, based on physiological age). The described methodology and results provide quantitative insight into E2 dynamics and serves as a model for future endocrinological studies in poultry reproduction.

Key words: reproduction, photorefractoriness, sexual maturity

INTRODUCTION

Reproduction in broiler breeders has become a field of increased interest as continuing selection pressure for growth over the past decades has resulted in reproductive challenges specifically related to overfeeding, such as erratic oviposition and defective egg syndrome (Jaap and Muir, 1968; Eitan et al., 2014). Yet the underlying endocrinological mechanisms of reproduction have not

yet gained full attention. In poultry, estradiol-17β (**E2**) is the main circulatory hormone involved in reproduction and the process of sexual maturation. E2 is produced within the theca cells of the small follicles in the ovary of the prepubertal hen in response to luteinizing hormone (**LH**; Senior and Furr, 1975; Robinson and Etches, 1986). During reproductive development, E2 stimulates the hypothalamus and pituitary to respond to progesterone (Wilson and Sharp, 1976). E2 is also involved in the development of the reproductive tract (Etches, 1990) and physiological processes outside of the reproductive tract required for egg production, such as synthesis of the majority of yolk components in the liver (Deeley et al., 1975) and blood calcium homeostasis critical for eggshell synthesis and medullary bone formation (Etches, 1987; Dick et al., 2003; Wistedt et al., 2014).

In broiler breeders, the process of sexual maturation before the onset of reproduction is affected by rearing photoperiod and BW or feed allowance before and after

photostimulation. However, many of the underlying physiological and metabolic mechanisms as well as the dynamics of E2 remain unclear (Bédécarrats et al., 2016). Previous studies showed that *ad libitum* feeding during the rearing period resulted in higher E2 levels and an earlier age at first egg (**AFE**) compared to restricted feeding (Bruggeman et al., 1998; Onagbesan et al., 2006). Yet, once E2 reached its peak level, feed restricted hens had higher E2 levels than *ad libitum* fed birds (Onagbesan et al., 2006). Feeding broiler breeder hens *ad libitum* also resulted in less hatching eggs compared to restricted feeding (Robinson et al., 1991; Bruggeman et al., 1999). Plasma E2 concentration was increased by increasing BW or feed allowance after photostimulation in feed-restricted pullets and peak E2 levels occurred earlier (Renema et al., 1999b). However, others concluded that regardless of feed restriction level or genetic background, an equivalent increase in E2 levels started 3 to 4 wk prior to the onset of lay (Eitan et al., 1998).

Although rearing photoperiod is a major factor in the timing of sexual maturation in broiler breeders, no literature could be found investigating the effect of rearing photoperiod on E2 levels in broiler breeder hens during puberty. Longer rearing photoperiods (>13 h) have been known to decrease the dissipation rate of the photorefractory state and consequently, increase the age at sexual maturation (Payne, 1975; Lewis et al., 2003, 2004; Lewis, 2006). Further, the effect of rearing photoperiod on AFE is dependent on BW (van der Klein et al., 2018). In hens with increased BW, age at sexual maturity did not differ between hens under 8L:16D and 10L:14D rearing photoschedules. However, for hens on a Standard BW, a 12L:12D rearing photoschedule delayed sexual maturity compared to an 8L:16D rearing photoschedule (van der Klein et al., 2018). The mechanisms behind these results are still unknown.

One of the challenges with the current published literature is that E2 levels in broiler breeders have been sometimes compared at the same chronological age (Onagbesan et al., 2006), sometimes relative to the E2 peak (Renema et al., 1999b), or sometimes at the same physiological age, i.e., relative to AFE (Eitan et al., 1998). The disadvantage of using a chronological comparison is that at a given chronological age some birds may be sexually mature, whereas others have not yet started to sexually develop or laid their first egg. Using chronological age, differences between E2 levels of experimental groups primarily reflects the different proportions of birds that have sexually matured in the experimental groups. Synchronizing treatments relative to their E2 peak creates the risk that the peak of the E2 levels can be easily missed. Daily variation in E2 levels, small errors or variation of the sample analysis, or insufficient sampling frequency can all result in missing peak E2 levels. The challenge with using AFE as a reference for physiological age is that data points of each individual might not be presented at each physiological age as hens may widely differ in AFE. The only way around this would be to collect samples daily for a prolonged period, requiring more invasive sampling methods like intravenous catheterization, which may reduce animal welfare. In addition, higher sampling frequencies and analytical tests are also more expensive. Therefore, there is a need for a more holistic and integrative way to study repeated measures of E2 levels to compare treatment effects. In human medicine, modeling techniques have been used to describe and study dynamics in endocrinological data (for example, Brown (1983)), but this approach is novel in the field of poultry science. Comparing treatments in this way, would also not depend on high sampling frequencies.

Clear understanding of the effects of BW and rearing photoperiod on the reproductive development of broiler breeder hens is needed to understand the challenges related to their reproductive performance. Therefore, the objective of this study was twofold. First, a model was developed as a tool to compare E2 levels in a holistic and integrative manner and to provide scientific insight into E2 profiles and dynamics. Second, the effect of rearing photoperiod and BW on plasma E2 levels in broiler breeders was interpreted using this novel methodology.

MATERIALS AND METHODS

Experimental Design

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). The experiment was a completely randomized design conducted as a 2×3 factorial arrangement of treatments with pullets reared either on a breeder-recommended target BW curve (Standard; Aviagen, 2016) or an accelerated target BW curve reaching the 21 wk target BW at 18 wk (High), and maintained under 8L:16D, 10L:14D, or 12L:12D photoschedules during rearing. The High target BW was 22% higher than the Standard target BW at 21 wk of age.

Animals and Housing

The experimental protocol was similar to that previously described by van der Klein et al. (2018). In brief, Ross 708 broiler breeder chicks (n = 180; provided by Aviagen, Huntsville, Alabama, USA) were neck tagged for individual identification and randomly allocated in six environmentally controlled rooms measuring 3.8×2.2 m (30 chicks per room). Birds were housed on the floor throughout the experiment and floors of the rooms were covered with wood shavings. Temperature was 34°C at d 0 and decreased with 0.5°C per d till d 30. Temperature was maintained at 19°C throughout

the experiment. Each room was equipped with one precision feeding (**PF**) system (Zuidhof et al., 2016, 2017), an automated computerized individual feeder for poultry. Water was provided *ad libitum* during the entire experiment. From d 0 to 16, birds were trained to use the PF system and were fed *ad libitum*. At d 16, birds were identified with a radio frequency identification (RFID) tag and randomly assigned to either the Standard or High BW treatment, such that approximately half of the birds per room were assigned to either target BW curve. From d 16 onwards all birds were fed individually. Thus, each bird was an experimental unit. The PF system identified individual birds through their RFID tag and controlled individual feed intake to achieve and adhere to the assigned target BW curves. BW of individual birds was measured with a scale inside the PF system and compared in real-time with the target stored in the computer database of the PF system. Birds were allowed access to feed for a duration of 45 s when their BW was lower than their treatment target BW at the moment they entered the PF system. When their measured BW was equal to or higher than their treatment target, birds were ejected from the PF system without access to feed. Birds had access to the PF system 24 h/d, hence were fed frequently throughout, depending on the visit activity of the bird. Treatment BW targets were updated on an hourly basis. At the start of the experiment, pairs of rooms were randomly assigned to either an 8L:16D, 10L:14D, or 12L:12D rearing photoschedule. For the first 2 d, a 23L:1D photoschedule was used to ensure full access to water and feed, after which the photoperiod was decreased by 2 h/d until the treatment photoschedule was reached. Hens from all treatments were photostimulated at wk 21 with a single abrupt step to 16L:8D. The light source (60% red, 20% green, and 20% blue LED light bulbs; PGR-11, AgriLux, Cambridge, ON) provided 8 lux during rearing and 25 lux during the laying phase. For the first 3 wk, chicks received a standard wheat based starter diet (2900 AME, 19% CP, 1.1% Ca); from wk 4 to wk 23 pullets received a wheat and barley-based grower diet (2589 AME, 14.2% CP, and 0.9% Ca); from wk 23 to wk 34 hens received a wheat-based peak layer diet (2689 AME, 15.0% CP, and 3.3% Ca).

Data Collection

A detailed description of data collection methods can be found in van der Klein et al. (2018). In brief, the PF station recorded and controlled BW individually on a per visit basis after d 16. Floor eggs could not be attributed to individual hens because hens on different BW treatments were housed in the same room. Therefore, prior to oviposition, cloacae of all hens were palpated daily just after lights turned on to detect the presence of a hard-shelled egg in the shell gland to measure AFE. The majority of the birds on the 8L:16D photo-

schedule treatment had entered lay by wk 36 thus, from 36 wk onward, daily palpation was performed every second wk.

Hormone Analysis

From wk 20 to 28, weekly blood samples (2 mL) were taken from the brachial vein of six randomly selected birds per BW × photoschedule treatment interaction. Blood samples were taken 1 to 3 h after lights were turned on and weekly repeated on the same birds. Blood samples were collected in 4 mL sodium heparin blood vacutainer. Immediately after collection plasma was recovered by centrifugation at 1244 g-force at 4°C for 15 min. Plasma samples were stored at −20°C till extraction. Hormone extraction was carried out according to the method suggested by Baxter et al. (2014). Thawed plasma samples were diluted with ethanol at a 1:5 (plasma:ethanol) ratio. Samples were vortexed, centrifuged for 5 min at 1,800 g-force at 20°C and frozen at −80°C. The organic (ethanol) phase was recovered, transferred into new tubes, and dried using a SpeedVac (Thermo Savant SpeedVac SC210A Centrifugal Evaporator, Thermo Scientific, USA). Samples were reconstituted in half the original volume with assay buffer and stored at −20°C until assay. Plasma E2 was measured in thawed extracted plasma samples using the DetectX 17β-Estradiol, Enzyme Linked Immunosorbent Assay kit (K030-H5, Arbor Assays®, USA) according to the manufacturer's instructions. Sensitivity of the kit was 39.6 pg/mL and cross reactivity of was 0.73% for estrone, and less than 0.10% for estrone sulfate, progesterone, testosterone, 5α-dihydroprogesterone, cortisol, corticosterone. Briefly, 50 μL of each extracted plasma sample was added in duplicate in individual wells of microtiter plates coated with goat anti-rabbit IgG antibody. Subsequently, 25 μL of DetectX estradiol conjugate to horseradish peroxidase and 25 μL of DetectX estradiol antibody (anti-E2 antibody) were added to each well. Reagents and plasma samples were mixed and incubated at room temperature while shaking for 2 h. Thereafter, wells were aspirated and washed 4 times with 300 μL of wash buffer. Next, 100 μL of tetramethyl benzidine substrate was added to each well and left to incubate at room temperature for 30 min. Finally, 50 μL of stop solution (sulphuric acid) was added to terminate the reaction. The optical density was measured with a microplate spectrophotometer at 450 nm (Molecular Devices, California, USA). The standard curve and samples were plotted and analyzed using SoftMax® Pro (Version 5, Molecular Devices, USA). The intra and inter assay coefficients of variation were 5.5 and 13.7%, respectively.

Design of Models

Two mixed non-linear models were considered using either the complete E2 dataset or a subset of
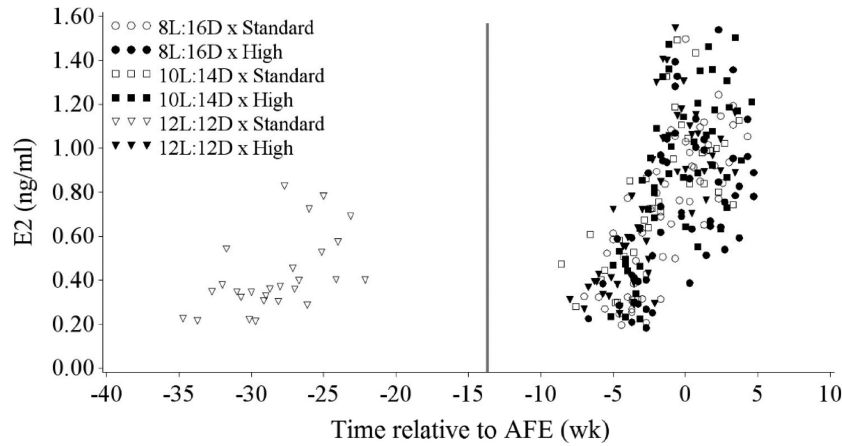
Figure 1. Estradiol-17β (E2) levels in broiler breeder hens relative to individual age at first egg (AFE, time = 0 wk) between wk 20 and 28 and fed to achieve either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule. Individuals to the left of the grey vertical reference line at 14 wk before AFE were considered photorefractory, individuals on the right of the grey vertical line were considered photosensitive.

all E2 data. The subset of E2 data only contained hens for which AFE was within 100 days of photo-stimulation and is referred to as photosensitive hens. The reason for distinguishing hens based on photosensitivity was that visual analysis of the data (Figure 1) showed that photosensitive and non-photosensitive hens were distinguishable based on time relative to AFE, which influenced the fit of the models. Hens that did not commence egg production during the entire experiment were excluded from all analysis (3.3, 18.1, and 37.6% of the Standard BW hens on the 8L:16D, 10L:14D, and the 12L:12D photoschedule, respectively), all hens on the High BW treatment commenced egg production (van der Klein et al., 2018). Both models described E2 levels as a function of age in wk and were based on a Gompertz growth curve (Tjørve and Tjørve, 2017). The models were specified as follows:

Chronological age model:

$$E_{it} = E_b + (E_m - E_b) * e^{-e^{-b\ (t-\ (t_{inf}+\ u_i))}} + \varepsilon_i \qquad (1)$$

Physiological age model:

$$E_{it} = E_b + (E_m - E_b) * e^{-e^{-b\ (t-AFE_i-(t_{inf}+u_i))}} + \varepsilon_i \qquad (2)$$

Where E_{it} = plasma E2 level at age t (ng/mL) of hen i; E_b = prepubertal E2 baseline (ng/mL); E_m = asymptotic E2 level after sexual maturation (ng/mL); b = rate coefficient; t = age (wk); t_{inf} = E2-inflection point (age (wk) at which the increase in E2 occurs at the greatest rate) [1] or time (wk) before AFE at which the increase in E2 occurs at the greatest rate [2]); AFE_i = age at first egg (wk) of hen i; u_i = hen-related random term; ε_i = residual error of hen i. The error term u accounted for temporal variation associated with each

hen; variance parameters u $\sim$ N(0, V_u) and $\varepsilon \sim$ N(0, V) were estimated in the regressions. Model [1] used chronological age and model [2] used physiological age; the latter adjusted the age at sample collection by individual AFE. The time after photostimulation at which the E2 increase occurred at the highest rate (the E2-inflection point) was calculated for each individual hen as the difference between individual E2-inflection point and the age at photostimulation. The duration between individual E2-inflection point and AFE was calculated as the difference between individual E2-inflection point and individual AFE.

Statistical Analysis

Differences among treatments and least squares mean estimates of variables included in the models were evaluated using the MIXED procedure of SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012). Tukey's range test was used to compare treatment means and were considered significant at $P \leq 0.05$. Bird was the experimental unit. Non-linear regressions were performed using the NLMIXED procedure of SAS (SAS Institute), which used maximum likelihood and allowed specifying a distribution of random effects, which were clustered by subject (bird). The Bayesian information criterion (BIC) and the Akaike information criterion (AIC) were used to evaluate the fit of the models; lower BIC or AIC values mean a better fit. Mean squared error (MSE) and R-squared values were also calculated with the following formulae:

$$MSE = \frac{1}{n} \sum_{i=1}^{n} \left(Y_i - \hat{Y}_i\right)^2$$

$$R^2 = 1 - \frac{\sum_i \varepsilon_i^2}{\sum_i (y_i - \bar{y}_i)^2}$$

Table 1. Functional specifications, coefficients, and fit statistics criteria of the modified Gompertz models describing estradiol-17β (E2) levels as a function of age.

	Chronological age model [1]						Physiological age model [2]					
Equation	$E_{it} = E_b + (E_m - E_b) * e^{-e^{-b\,(t-(t_{inf}+u_i))}} + \varepsilon_i$						$E_{it} = E_b + (E_m - E_b) * e^{-e^{-b\,(t-AFE_i-(t_{inf}+u_i))}} + \varepsilon_i$					
	All hens			Photoresponsive hens			All hens			Photoresponsive hens		
Parameter[1]	Estimate	SEM	P-value	Estimate	SEM	P-value	Estimate	SEM	P-value	Estimate	SEM	P-value
E_b	0.35	0.038	<0.001	0.33	0.050	<0.001	0.42	0.025	<0.001	0.43	0.031	<0.001
E_m	1.09	0.039	<0.001	1.07	0.041	<0.001	1.06	0.023	<0.001	1.06	0.024	<0.001
b	0.86	0.219	<0.001	0.98	0.290	0.002	2.58	0.541	<0.001	2.56	0.516	<0.001
t_{inf}	22.61	0.347	<0.001	21.91	0.255	<0.001	−2.37	0.131	<0.001	−2.37	0.131	<0.001
V	0.07	0.006	<0.001	0.08	0.007	<0.001	0.07	0.006	<0.001	0.07	0.006	<0.001
V_u	2.28	0.758	0.005	0.22	0.275	0.435	0.10	0.049	0.060	0.10	0.062	0.139
Criterion												
BIC[2]		123.9			96.2			65.0			75.8	
AIC[3]		115.2			88.2			56.4			67.8	
R-squared		0.27			0.05			0.27			0.17	
MSE[4]		0.065			0.074			0.065			0.069	

[1]E_{it} = plasma E2 level at age t of hen i (ng/mL); E_b = prepubertal E2 baseline (ng/mL); E_m = asymptotic E2 level (ng/mL); b = rate coefficient; t = age (wk); t_{inf} [1] = E2-inflection point (age (wk) at which the increase in E2 occurred at the greatest rate); t_{inf} [2] = time before AFE at which the increase in E2 occurred at the greatest rate; AFE_i = age at first egg (wk) of hen i; u_i = hen related random term (wk); ε_i = residual error of hen i (ng/mL). The error term u accounted for temporal variation associated with each hen; variance parameters u $\sim$ N(0, V_u) and $\varepsilon \sim$ N(0, V) were estimated in the regressions.
[2]Bayesian information criterion; smaller values indicate a better fit of the model.
[3]Akaike information criterion; smaller values indicate a better fit of the model.
[4]Mean-Squared Error.

RESULTS AND DISCUSSION

Animal Performance

Detailed description of animal performance such as feed intake, BW, and AFE was reported previously in van der Klein et al. (2018). As it relates to the current experiment, some AFE results are summarized in this section. In the High BW treatment, AFE did not differ between hens on the 8L:16D and 10L:14D rearing photoschedules (173.5 vs. 171.8 d, respectively), and the 12L:12D treatment delayed AFE (210.4 d). In the Standard BW treatment, the 12L:12D rearing photoschedule delayed sexual maturity compared with the 8L:16D rearing photoschedule (266.1 vs. 180.4 d, respectively), and the 10L:14D treatment was intermediate (211.7 d). Overall, hens on the High BW treatment reached AFE earlier compared to hens on the Standard BW treatment (185.2 vs. 219.4 d).

Model Evaluation

The described modeling methodology provides insight into E2 dynamics. In addition, it is able to extract value from less data or measuring points than previously possible. For all models convergence was achieved. R-squared values were relatively low (Table 1). Model [2] fits the data better, as BIC and AIC values are lower, either when all hens, or when only photoresponsive hens were included in the data. Interestingly, when model [1] was used, there was clearly an advantage of only including the photoresponsive hens and with model [2] there was benefit in using information from all hens to fit the model. This was due to model [2] correcting for the fact that the photorefractory hens had a delayed AFE. To moderate fluctuations in E2 concentrations between weeks, 2 wk moving averages have been used in previous studies (Eitan et al., 1998). The current models provided the advantage that these fluctuations were accounted for by the error term 'u'. This enabled the use of all individual measurements instead of averages.

Figure 2 shows a visualization of the fitted model parameters from Table 1. Here it can be seen that model [2] estimated a steeper increase in E2 plasma concentration compared to model [1] where a more gradual increase in E2 is estimated (associated with the respectively higher and lower b values in Table 1). A more gradual increase could indicate a slower development of the E2 producing capacity of the small follicles in response to LH, a steeper increase indicates a fast development and response. Often, the published literature presented E2 averages of individuals at different physiological ages within one treatment group in figures, which does not represent the true individual dynamics of the E2 increase. The graph of model [2] shows visual similarities in rate of increase with results from Eitan et al. (1998) relative to AFE. Therefore, it is hypothesized that model [2] reflects the actual individual dynamics of E2 increase more closely than model [1]. Renema et al. (1999b) indicated that there was a slower rate of change in the establishment of elevated E2 levels in low BW birds compared to high BW birds as they had a reduced rate of change in E2 levels between photostimulation and peak E2 level (5.81 vs. 9.78 pg/mL/d, respectively). In the current study, a different approach was taken, in which the rate of increase (parameter b) was assumed to be similar for all birds, as the available data was limited.
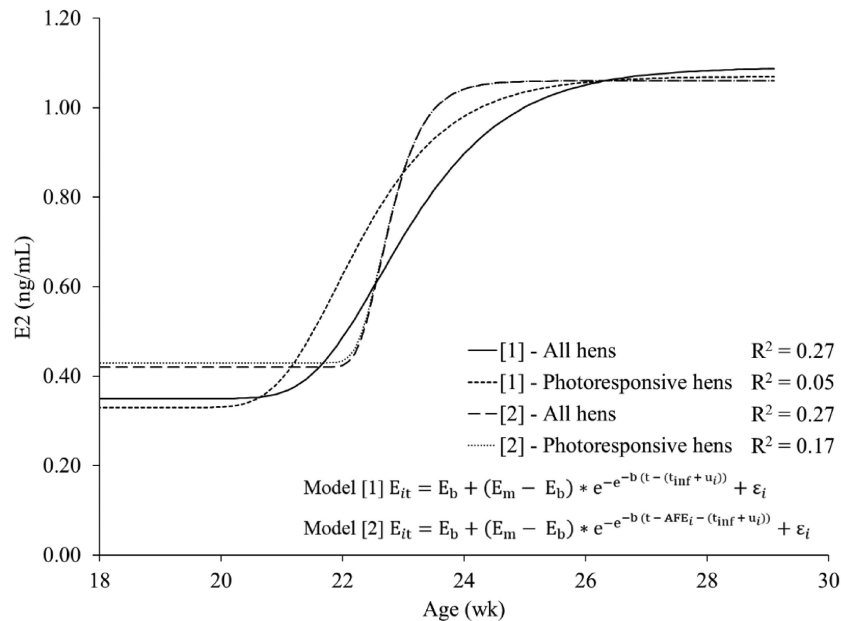
Figure 2. Comparison between predicted estradiol-17β (E2) levels in broiler breeder hens, modeled by a modified Gompertz curve including chronological age (Model [1]) or physiological age (age relative to age at first egg, Model [2], the average age at first egg of 25 wk was used), including all hens or the subset of photoresponsive hens who laid their egg within 100 d of photostimulation. E_{it} = plasma E2 level at age t of hen i (ng/mL); E_b = prepubertal E2 baseline (ng/mL); E_m = asymptotic E2 level (ng/mL); b = rate coefficient; t = age (wk); t_{inf} [1] = E2-inflection point (age (wk) at which the increase in E2 occurred at the greatest rate); t_{inf} [2] = time before AFE at which the increase in E2 occurred at the greatest rate; AFE_i = age at first egg (wk) of hen i; u_i = hen related random term (wk); ε_i = residual error of hen i (ng/mL). The error term u accounted for temporal variation associated with each hen; variance parameters u $\sim$ N(0, V_u) and $\varepsilon \sim$ N(0, V) were estimated in the regressions.

The physiological reference point in model [2] was the E2-inflection point. This was advantageous over using peak E2 levels as a physiological reference (Renema et al., 1999b), as the peak in E2 levels can be easily missed if sampling is not performed frequently enough and consequentially information from the individual bird cannot be used for comparisons.

Differences in baseline E2 levels and asymptotic E2 levels were assumed to be the same for all birds. Baseline E2 levels were estimated between 0.33 and 0.43 ng/mL, and asymptotic E2 levels were estimated between 1.06 and 1.09 ng/mL. These values are comparable to some previous studies investigating E2 levels around the same age (Bruggeman et al., 1998; Rodriguez, 2017), but higher than others (Renema et al., 1999b; Sun et al., 2006). However, as E2 analysis techniques vary between studies, direct comparisons of E2 concentrations between studies hold little value as differences could be associated with different methods, for example comparing ELISA with radioimmunoassay, the sensitivity and specificity of different antibodies used, or analysis on ethanol-extracted samples vs. non-extracted samples. Future experiments could explore inclusion of additional random variables to the rate parameter, or the baseline and asymptotic E2 level parameters in the presented models. These experiments could also evaluate whether BW or rearing photoperiod treatments affect the variation in these parameters.

Treatment Comparisons on Timing of the E2-Inflection Point

The current mathematical methodology allowed for meaningful comparison of the timing of the E2-inflection point, instead of visually interpreting the pattern of increase as was done by Eitan et al. (1998). For treatment comparisons of the timing of the E2-inflection point, all hens were included in the analysis (both models), as hens on the 12L:12D rearing photoschedule selected for E2 analysis were photorefractory at photostimulation and treatments could otherwise not be compared (Figure 1). This is an advantage of the current methodology, as previous studies would have had to exclude data from the 12L:12D treatment.

The effect of BW on the duration between photostimulation and the E2-inflection point depended on rearing photoperiod (Table 2), and were in line with differences in AFE. For model [1], in the Standard BW treatment, the 12L:12D rearing photoschedule had a prolonged period between photostimulation and the E2-inflection point compared to the 10L:14D and 8L:16D rearing photoschedule, whereas in the High BW the period between photostimulation and the E2-inflection point was prolonged in the 12L:12D rearing photoschedule compared to the 10L:14D rearing photoschedule, but the 8L:16D was intermediate. In both models, hens on the Standard BW treatment had a longer period between photostimulation and the E2-inflection point compared to hens on the High BW treatment

Table 2. Time between photostimulation[1] (PS) and the E2-inflection point (PS to E2 increase) and time between E2 increase and age at first egg (E2 increase to AFE) of hens[2] fed to achieve a High or Standard BW[3] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS) as predicted by modified Gompertz curves including chronological age or physiological age, relative to age at first egg.

	Model		Chronological age				Physiological age			
Effect	BW	RPS	PS to E2 increase	SEM	E2 increase to AFE	SEM	PS to E2 increase	SEM	E2 increase to AFE	SEM
			wk							
BW	High		1.04^b	0.162	2.85^b	0.246	1.50^b	0.318	2.38	0.034
	Standard		2.74^a	0.198	10.67^a	0.301	11.03^a	0.390	2.37	0.042
RPS		8L:16D	1.44^b	0.198	2.21^c	0.301	1.35^b	0.390	2.29^b	0.042
		10L:14D	0.91^b	0.222	3.22^b	0.336	1.67^b	0.436	2.46^a	0.047
		12L:12D	3.30^a	0.243	14.86^a	0.368	15.78^a	0.477	2.39^{a,b}	0.051
BW × RPS	High	8L:16D	1.07^{b,c}	0.280	2.15^c	0.425	0.93^c	0.551	2.29	0.059
		10L:14D	0.39^c	0.280	3.02^{b,c}	0.425	0.94^c	0.551	2.47	0.059
		12L:12D	1.66^b	0.280	3.39^b	0.425	2.65^b	0.551	2.40	0.059
	Standard	8L:16D	1.81^b	0.280	2.27^{b,c}	0.425	1.78^{b,c}	0.551	2.29	0.059
		10L:14D	1.44^b	0.343	3.42^{b,c}	0.521	2.40^{b,c}	0.675	2.46	0.072
		12L:12D	4.96^a	0.396	26.32^a	0.602	28.91^a	0.779	2.37	0.083
Source of variation			P-value							
BW			<0.001		<0.001		<0.001		0.856	
RPS			<0.001		<0.001		<0.001		0.034	
BW × RPS			0.001		<0.001		<0.001		0.976	

[a–c]LSMeans within a column and effect lacking a common superscript differ ($P \leq 0.05$).
[1]Photostimulation occurred at wk 21.
[2]Hens that did not commence egg production before wk 55 were excluded from the analysis.
[3]Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

(2.74 vs. 1.04 wk for model [1] and 11.03 vs. 1.5 wk for model [2], respectively). Renema et al. (1999b) suggested that some initial sexual maturation can occur prior to photostimulation due to a larger population of small white follicles (<1 mm in diameter) in *ad libitum* fed birds compared to feed restricted birds. In addition, Yu et al. (1992) reported that small white follicles from *ad libitum* fed birds produced androstenedione, a precursor for E2 production, compared to feed restricted birds (3 vs. 2 ng/mL, respectively). However, they were unable to detect differences in E2 production in small white follicles from *ad libitum* and feed restricted birds. Also, Bruggeman et al. (1998) reported that *ad libitum* fed pullets had a 3.4 fold higher plasma E2 concentrations at week 16 compared to pullets that had been feed restricted during rearing. Onagbesan et al. (2006) reported that E2 levels prior to peak were 1.9 fold higher in *ad libitum* fed birds compared to feed restricted birds, and that peak plasma E2 levels occur about 3 wk earlier in birds fed *ad libitum* compared to feed restricted birds.

Some of the underlying mechanisms of the previous described differences between *ad libitum* and restricted fed birds may originate from the fat pad, as *ad libitum* fed birds have a higher fat pad weight (Renema et al., 1999a). Differences in mRNA and protein expression in visceral fat pointed to a direct communication of the chicken fat pad with the reproductive system (Bornelöv et al., 2018). Protein and mRNA expression differentiated between laying hens and broiler breeders in the first week of lay (LCAT, LECT2, SERPINE2, SFTP1, ZP3, APOV1, VTG1, and VTG2) and for *ad libitum* fed or 24 h feed-deprived

birds (NAMPT, SFTPA1, and ZP3). In addition, the adipokinetic response of the fat pad to feed restriction could also directly stimulate the hypothalamus–pituitary–gonadal axis. Unfortunately, in the current study we did not evaluate fat pad weight at photostimulation, yet we expect that the High BW birds would have had a heavier fat pad compared to Standard BW birds. A simple ANOVA of the current data at photostimulation showed that E2 levels were higher in High BW birds compared to Standard BW birds (P = 0.032, 0.384 ng/mL vs. 0.287 ng/mL, respectively). As High BW birds also matured faster, this could indicate that an as-yet unknown metabolic signal primed the hypothalamus–pituitary–gonadal axis and provided the High BW birds the ability to respond faster to photostimulation compared to Standard BW birds (Wilson and Sharp, 1976). Interestingly, at the wk before photostimulation no significant difference was found between E2 levels of High and Standard BW birds. This could mean that the metabolic signal is only released after photostimulation.

There was a larger difference in the duration between photostimulation to the E2-inflection point between the two BW treatments in model [2] compared to in model [1] (9.53 wk vs. 1.7 wk, respectively). As the period from photostimulation to AFE was determined on an individual basis, model [2] seemed to capture most of the treatment difference in the timing of the E2-inflection point in the period between photostimulation and the E2-inflection point. Model [1] captured treatment differences both in the period between photostimulation and the E2-inflection point and the period between the E2-inflection point and AFE,

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with a larger portion of the difference in the latter period.

Results from model [2] quantitatively inferred that the E2-inflection point occurred consistently around 2.4 wk before AFE, which is similar to the visual observations of Eitan et al. ([1998](#)). They concluded that E2 levels remained low with some fluctuations until about 3 or 4 wk prior to AFE, after which a sharp increase occurred. The time difference between their study and our result of 2.4 wk is explained by that previous authors focused on the start of the increase, instead of the moment at which the E2 increase occurs at the highest rate.

The effect of photoperiod on timing of the E2-inflection point reflected in model [1] shows that the 12L:12D rearing photoschedule extended the period between the E2-inflection point and AFE compared to the 8L:16D rearing photoschedule, with the 10L:14D being intermediate (Table [2](#)). Interestingly, model [1] shows a much larger effect compared to model [2]. In model [2], there was only a small effect of rearing photoperiod on the duration between the E2-inflection point and AFE, and no difference between BW treatments. Hens on the 8L:16D rearing photoschedule matured faster after the E2-inflection point compared to hens on the 10L:14D rearing photoschedule (2.29 wk vs. 2.46 wk, respectively). This contrasts with Renema et al. ([1999b](#)), who demonstrated that the alignment of the E2 profiles for each bird with the physiological event of peak E2 level in their experiment produced similar patterns for all their treatments. Although no integrative quantitative analysis was performed, Renema et al. ([1999b](#)) hypothesized that once pubertal ovary development commences, it proceeds at a predictable rate. The current result shows a difference of 1.4 d between the 8L:16D and 10L:14D photoschedule treatments, yet this may not be of any practical significance. The 12L:12D rearing photoschedule was intermediate between the 10L:14D and the 8L:16D treatment, for which an explanation could not be found. Further research is needed to determine whether or not rearing photoperiod influences the rate of sexual development after the E2-inflection point.

In this study, hens were individually fed multiple times a day with a PF system, whereas most studies use the standard practice of daily or skip-a-day feeding during rearing and daily feeding after photostimulation. Wiggle ([2008](#)) concluded that the frequency of feeding can affect ovarian development when comparing daily to skip-a-day feeding after photostimulation, yet this was not related to differences in onset of E2 production. Still, the latter study only used a comparison between treatments based on chronological age, which may have confounded the conclusion. It is interesting to note that 10% of skip-a-day hens had produced eggs at wk 26 compared to 60% of the daily fed hens in the latter study. AFE was not reported, but it could be inferred from egg production results that hens on skip-a-day feeding were delayed in their onset of lay. This

would mean that increasing feeding frequency advances the onset of lay.

CONCLUSIONS

To our knowledge, this is the first time a mathematical methodology has been developed to describe and predict differences in E2 profiles and dynamics in broiler breeders. The model based on chronological age predicated that the duration between the E2-inflection point and AFE was longer in the Standard BW treatment compared to the High-BW treatment, whereas the model based on physiological age predicated that the duration between photostimulation and the E2-inflection point was longer in the Standard BW treatment compared to the High-BW treatment. In addition, the peak rate of E2 increase occurred consistently around 2.4 wk before AFE. The described methodology provides an example for other studies into endocrinological dynamics in poultry reproduction. The methodology is able to create value from less datapoints than previously possible and showed scientific insight into the dynamics of E2 concentration during sexual maturation in response to BW and rearing photoperiod. As the methodology is able to identify individual dynamics in E2 plasma concentration these individual parameters could potentially serve breeding purposes.

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