Controlling the partitioning of energy intake in meat-type chickens

by

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ABSTRACT

Meat-type chickens have been bred for prioritizing energy partitioning to rapid gain and lean tissue growth, which required feed restriction to be commonly applied in the industry to optimize egg production. This thesis studied the effects of controlling energy intake on energy partitioning to maintenance, growth, and reproduction in meat-type chickens (broilers and broiler breeders). Precision feeding was used as a tool to control feed intake or body weight (BW) and collect the required feed intake and BW data of each individual bird. A modelling methodology was compared with the comparative slaughter technique (CST; industry standard) in broilers to estimate energy partitioning to maintenance and growth. Maintenance and growth were estimated by CST and with a non-linear mixed model explaining daily metabolizable energy intake as a function of metabolic BW and daily gain. The estimated values from the model approached the values estimated by the CST.

BW and lighting both have a large effect on sexual maturation and egg production in broiler breeders, but their interaction was hitherto unknown. It was hypothesized that an increase in BW above the breeder-recommended target and an advanced age at which photoperiod would be increased (photostimulation) would advance the onset of lay (sexual maturity) and increase egg production. Sexual maturity was advanced and maturation interval between photostimulation and sexual maturity was shorter for hens with higher BW compared to hens on the breeder recommended BW target. Hens photostimulated at week 21 matured earlier and had a higher egg production compared to hens photostimulated at week 18.

It was also hypothesized that the effect of the light schedule during the rearing phase would depend on BW. Therefore, two growth curves and three rearing photoperiod treatments were compared. The age at sexual maturity did not differ between hens on an 8 h or 10 h rearing light schedule at

a higher BW target, but the 12 h rearing photoperiod delayed sexual maturity at the higher BW. Hens at the breeder recommended target had delayed sexual maturity with the 8 h, 10 h, and the 12 h treatments. All hens on the higher BW treatment laid at least one egg before the end of the experiment. Almost 40% of the hens on the breeder recommended target and the 12 h treatment did not commence egg production during the experiment.

The plasma concentration of estradiol-17β (E2), an important hormone involved in sexual maturation, was measured to study the underlying cause of the differences between treatments. Two models were developed based on modified Gompertz curves, to describe E2 level as a function either chronological or physiological age (i.e. relative to age at first egg). Hens on the breeder recommended BW target had a longer period between photostimulation and the age at which E2 increased at the highest rate compared to hens on a higher BW target. Hens on the 12 h rearing photoperiod treatment had a longer period between photostimulation and the age at which E2 increased at the highest rate compared to hens on the 8 h and 10 h in both BW target treatments.

It was hypothesized that hens on the shorter rearing photoperiod and with decreased BW, would be more energetically conservative. The model for energy partitioning in broiler breeders included a random effect for individual maintenance requirement and age-related maintenance requirements and provided a biologically sound estimation of life-time energy partitioning. Although it was estimated that hens on the recommended BW target with a 12 h rearing photoperiod were most energetically conservative, their egg production was the poorest.

This thesis concludes that current recommended breeder BW could be too low for optimal sexual maturation after photostimulation in precision fed broiler breeders. Even when BW variation is minimized through precision feeding, early photostimulation is not recommended. Increased BW

partially counteracted the effect of longer photoschedules on sexual maturity in broiler breeders and that this effect depended on BW. The described modeling methodologies and results provide quantitative insight into E2 dynamics and energetic partitioning during the broiler breeder hens' life-time.

PREFACE

This thesis is an original work by Sasha A. S. van der Klein. Funding for the project described in Chapter 3 was provided by Danisco Animal Nutrition. Funding for the project described in Chapter 4 to 7 was provided by Alberta Livestock and Meat Agency, Ontario Ministry of Agriculture, Food and Rural Affairs, Canadian Poultry Research Council, Alberta Innovates Bio Solutions, Poultry Industry Council, Alberta Hatching Egg Producers, Canadian Hatching Egg Producers, and Alberta Chicken Producers. In-kind support for project described in Chapter 4 to 7 was provided by Xanantec Technologies Inc., Aviagen, and Thies Electrical Distributing Co.

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Chapter 6 of this thesis has been published as S.A.S. van der Klein, S.H. Hadinia, F.E. Robinson, G. Y. Bédécarrats, and M. J. Zuidhof, "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," in Poultry Science, volume 98, issue 10, pages 5137-5145. I was responsible for the data collection and analysis as well as the manuscript composition. S.H. Hadinia was responsible for the blood sample analyses. G.Y. Bédécarrats and F.E. Robinson contributed to critical review of the manuscript. M.J. Zuidhof served as advisors and provided critical review of the manuscript.

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The research projects received research ethics approval from the University of Alberta Research Ethics Board, Precision Broiler Breeder Feeding System, AUP00000121, June 9, 2014.

DEDICATION

"This thesis is dedicated to my parents and my sister, omdat jullie mijn honger naar wetenschap hebben gevoed en ondersteund met cultuur en creativiteit"

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I would like to thank my dad, Adriaan van der Klein, for feeding my inquiry skills throughout my childhood and unconditional support in all my education endeavours. I would like to thank my mom, Mariken Oltheten, for keeping an eye on the balance between life and my studies. During my degree I often had to think about your advice that "*you cannot do more than your best*". Academia sometimes makes you feel that your best is never enough, so with your wisdom you kept me going. I would like to acknowledge my sister, Olfje van der Klein, for allowing me to travel to almost the other side of the world for my PhD. It has been incredibly tough being physically so far away from my best friend, but modern communication technologies have made it bearable, including a significant number of questionable selfies.

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LIST OF ABBREVIATIONS

ADG	Average daily gain
AFE	Age at first egg
AIC	Akaike information criterion
ANOVA	Analysis of variance
BW	Body weight
BIC	Bayesian information criterion
CST	Comparative slaughter technique
CV	Coefficient of Variation
d	Day
E2	Estradiol-17β
EM	Egg mass
FCR	Feed conversion ratio
FSH	Follicle stimulating hormone
GE	Gross energy
GnIH	Gonadotropin inhibitory hormone
GnRH	Gonadotropin releasing hormone
HIF	Heat increment of feeding
HP	Heat production
HPG	Hypothalamic–pituitary–gonadal
LH	Luteinizing hormone
ME	Metabolizable energy
MEI	Metabolizable energy intake
ME_m	Metabolizable energy requirements for maintenance
MSE	Mean squared error
NE	Net energy
RME_m	Residual metabolizable energy requirement for maintenance
ME_g	Metabolizable energy requirement for BW gain
RE	Retained energy
RFI	Residual feed intake
RHP	Residual heat production

RPS	Rearing photoschedule
PF	Precision feeding
PS	Photostimulation
SEM	Standard error of the mean
wk	Week

CHAPTER 1.

General introduction

The fate of dietary energy needs to be understood to control resource partitioning in poultry. Feed accounts for a large proportion of the environmental footprint of chicken-meat production (Pelletier, 2008) and is generally understood to be associated with 60-70% of the cost of production. Therefore, this thesis addressed understanding the effect of controlling energy intake on the partitioning of metabolizable energy (ME) between maintenance, growth, and reproduction in meat-type chickens.

The modern broiler breeder hen is burdened with balancing her genetic potential growth and her production purpose for fertile egg production. After decades of intensive selection for fast and lean growth in broilers (Zuidhof et al., 2014), broiler breeders cannot self-regulate this balance. Under *ad libitum* feeding broiler breeders will prioritize growth, resulting in reproductive dysregulation and poor egg production (Richards et al., 2010). Hatching egg producers control feed allocation to their flock aiming at optimizing life-time productivity, which results in severe feed restriction in the rearing phase. In 1979, the breeder target body weight was 53% of the broiler at 6 weeks of age, which reduced to 27% in 2005 and 23% in 2019 (Renema et al., 2007; Aviagen, 2016, 2019). This has obvious implications for bird welfare, as birds display stereotypical behaviours and aggression due to hunger and competition at feeding time (Mench, 2002). It also poses a question on how broiler breeders partition their feed or energy intake under such severe feed restriction to maintenance, growth, and reproduction. Apart from controlling feed intake, lighting is the second most important management tool to optimize egg production in broiler breeders. Broiler breeders require short photoperiod exposure (less than 12 h per 24 h) followed by photostimulation (more than 12 h per 24 h) to reach sexual maturity (Lewis, 2006). Although management decisions such as feed allocation and lighting take place at a flock level, the production of a flock equals the sum of the production of the individual hens. Understanding the effect of the management decisions of feed intake and lighting on the ME partitioning between maintenance, growth, and reproduction on the individual level, will allow for more precise management on the flock level.

At the University of Alberta, a feeding system for poultry was developed to control and measure individual feed intake and body weight in a group housed setting (Zuidhof et al., 2017, 2018). This novel technology was used for all experiments in this thesis. The number of animals

was reduced and the statistical power of the experiments was increased, because individual measurements for body weight and feed intake were acquired through the PF system; therefore, the individual bird could be used as an experimental unit instead of the 'pen' in commonly used pen design experimentation, including multiple birds. The PF technology also refined the experiments by providing birds an environment closer to the industry standard (group housing) and more precise control over feed allocation decision criteria (feed intake (Chapter 3) or body weight (Chapter 4-7)).

Chapter 2.1 captures a short introduction into modeling, because models were used as a tool to understand energy partitioning (Chapter 3 and 7) and endocrine dynamics (Chapter 6). Chapter 2.2 provides an overview of the fate of energy in feed, discussing energy and energy partitioning. Chapter 2.3 provides a literature overview of the underlying causes of reproductive dysregulation in broiler breeders. The end of Chapter 2 concludes with the general problem statement and the overview of the objectives of this thesis. Chapter 3 evaluates, discusses, and validates an energy partitioning model and estimates the net energy value of feed in broilers. The experiments in Chapter 4 and 5 were run simultaneously. Chapter 4 discusses the interaction between target body weight and the age at photostimulation and their effects on egg production in broiler breeders. Chapter 5 discusses the interaction between target body weight and rearing photoperiod and their effects on egg production in broiler breeders; this chapter forms the basis of further analysis in Chapter 6 and 7. Chapter 6 provides an integrative model to investigate the effects of target body weight and rearing photoperiod on the circulatory levels of estradiol-17β. Chapter 7 employs a comparison of several models describing life-time energy partitioning in broiler breeders and shows the effect of photoperiod, body weight, and sexual maturity on energy partitioning. A synthesis and the conclusions of this thesis are provided in Chapter 8.

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CHAPTER 2.

Literature review

2.1 Modeling the truth

The University of Alberta's motto is '*quaecumque vera*', meaning 'whatsoever things are true'. Since the 17th century, the scientific method has been used to understand and iteratively redefine the 'things that are true' or 'the truth' (Gauch, 2003). The scientific method involves a process of observing, stating a hypothesis, testing a hypothesis, reaching a conclusion, and refining the hypothesis (Summers, 1998; Gauch, 2003). The truth can be viewed as an infinitely complex system, where, every iteration, the society gets closer to understanding 'the truth' using the scientific method. Methodological reductionism tries to provide an explanation of this system by studying its individual components and their interactions; reductionists see the whole system as the sum of its parts (Honderich, 2005). The reductionist approach has brought great advances in knowledge in poultry science (Etches, 1998), yet reductionism has been criticized to oversimplify the system or 'the truth' (Gallagher and Appenzeller, 1999). The relationship between parts of the system may not be a linear sum of its components (Kwon, 2019) and components may act in a synergy, inadequately described using the reductionist approach (Fardet and Rock, 2014). Mathematical modeling can be used as a tool to both represent and understand parts of the system by simplifying it (the reductionists approach), but also by linking and incorporating multiple parts of a system (the holistic or integrative approach). Models are also used to generate and test hypotheses, following the scientific method. There are two main types of mathematical models: 1) empirical or statistical models and 2) mechanistic or systems analytical models. An empirical model is a mathematical equation validated using data (Bonate, 2011), where parts of this equations can be intrinsic to the reality it describes (i.e. represent a real component of the system; Thakur, 1991). The mathematical equation can also be completely extrinsic and based on external behaviour of the system (i.e. unrelated to the components of the system). Mechanistic models describe the components and processes within a system, where the components and processes within the model always aim to represent reality. Differentiation between empirical and mechanistic models can be a topic of debate, as mechanistic models can originate from empirical work (Thakur, 1991). Models can also be static (single state) or dynamic (changing state over time) and deterministic (predicting a single outcome) or stochastic (predicting a range of outcomes; Bonate, 2011). Chapters 3, 6, and 7 of this thesis used models to understand the variance within

certain variables and to test hypotheses. The variance of a variable is a measure of variation and is defined as the expected value of the squared deviation from the mean of this variable: $\mathrm{Var}(X) = \mathrm{E}[(X - \mu)^2]$ also depicted as σ^2. The square root of the variance is known as the standard deviation (SD or σ).

The models in Chapters 3 and 7 explain energy partitioning, where they associate metabolizable energy (ME) intake with variation in individual and/or age-related maintenance requirements, growth, and egg production. These models were based on earlier work in this field and are additive mathematical equations with non-linear components (Byerly et al., 1980; Schulman et al., 1994; Romero et al., 2009b). The model in Chapter 3 uses empirical data to fit the equation, which does not change over time, and produces a single outcome. However, components of the model represent the reality of energy partitioning in living organisms (maintenance and growth). Therefore, the energy partitioning model in Chapter 3 can be classified as an empirical, static, deterministic model, where the equation components aim to represent reality. Chapter 7 explores an additional random term to the energy partitioning model, associated with individual age-related maintenance requirements. As age is a function of time, it can be argued that this model contains a dynamic characteristic. The energy partitioning model in Chapter 7 can therefore be classified as an empirical, static, deterministic model, where the equation parameters represent reality and the reality of a changing state is approached by including a dynamic characteristic to one of the parameters.

Chapter 6 uses two models to quantify the dynamics of circulatory levels of one of the major reproductive hormones in poultry, estradiol-17β (E2). Both equations were based on a four parameter Gompertz equation (Tjørve and Tjørve, 2017). The Gompertz equation was originally designed to describe the relationship between increasing death rate and age in humans (Gompertz, 1825) and was later applied to bovine body mass growth (Davidson, 1928). It has been further applied to many other fields of biology, as the parameters within the model are easily interpretable and the sigmoidal shape fits many biological processes. The models in Chapter 6 described the dynamics of E2 levels and parameters in the model are obtained fitting the model to collected data. Therefore, the models in Chapter 6 are empirical models. Both models include age, either chronological age or physiological age, and therefore are dynamic. The estradiol models in Chapter 6 can therefore be classified as empirical, dynamic, deterministic models, where the equations' parameters represent reality.

There is elegance and beauty in understanding the infinite complexity of reality from simple models. Model design and selection can therefore be viewed as an art within science (Bonate, 2011), as it is based on the balance between creativity and model simplicity, goodness of fit, bias, parameter relevance, and interpretability. Chapter 3 presents a comparison between a modelling approach and a well-established technique within animal science for energy partitioning, the comparative slaughter technique (CST). The model is tested on how well it represents the reality, or, how researchers have defined reality for many years by CST (Fraps, 1946). In Chapter 7, a model based on ideas and studies by other researchers was improved through increasing model complexity, resulting in improving goodness of fit. Chapter 6 resulted from an unexpected issue: no tools could be found in the literature to quantitatively compare the temporal dynamics of circulatory E2 levels between individual hens on different treatments. It required a creative modeling solution to be able to apply statistical inference to differences in dynamics of E2. The model is relatively easy to interpret as the parameters in the model coincide with some of the physiological characteristics and processes behind E2 level changes. However, the model in Chapter 6 does not incorporate the physiological peak in E2 level. Peak levels of circulatory gonadotropins and steroid hormones were previously used to compare treatments (Renema et al., 1999), as they represent a characteristic of endocrinological dynamics. However, other researchers failed to detect the E2 peak (Liu et al., 2004). This exemplifies that models do not have to be completely representing 'the truth', e.g. incorporate the E2 peak, to be right or wrong. The right model would bring one closer to 'the truth' and the wrong model would bring one further away from 'the truth'. As a wise man once said, there are 100 ways to do things right, but there are 1000 ways to do things wrong. If one cannot decide how to do things, it is suggested a model could provide insight in determining which way would be preferred.

Even though models do not have to completely represent 'the truth', they can be better or worse. Occam's razor or the law of parsimony is the fundamental basis of model selection. It states "*nunquam ponenda est pluralitas sin necessitate*", translated "entities should not be multiplied beyond necessity" (Tornay, 1938). The practical meaning is often inferred as the simplest solution is most likely the right one. In modelling, this means that given two models with the same outcome or the same fit, the simpler one should be preferred; or as Domingos (1999) revisited, the more comprehensible one should be preferred. However, the limitation of this fundamental basis is that

there are many different definitions of simplicity and greater simplicity does not necessarily lead to greater accuracy (Domingos, 1999).

Within each chapter in this thesis, several selection criteria were used to determine the most comprehensible or better model. The studies of C.F. Gauss in the early 1800 on the maximum likelihood and least squares theories were integral to the development, selection, and assessment of empirical models (Gauss, 1823). The mean square errors (MSE; Steel and Torrie, 1960), the Bayesian information criterion (BIC; Schwarz, 1978), and the coefficient of determination or the R-squared value (Steel and Torrie, 1960) are used in this thesis, and all base their origin in Gauss's work. The MSE is calculated as the mean of squared differences between the observed and predicted values and therefore includes information on the variance of the errors (Steel and Torrie, 1960; Bonate, 2011).

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

MSE includes squaring of each term and is therefore strongly influenced by outliers. In addition, the MSE does not give insight into the bias of the model or incorporate any measure of model complexity. The BIC solves some of these issues, and is calculated as indicated below (Schwarz, 1978).

$$\text{BIC} = \ln(n)\, k - 2\ln(\hat{L})$$

where $\hat{L}$ is the maximized value of the likelihood function of the model, n is the sample size, and k is the number of parameters estimated by the model. The BIC increases with the complexity of the model (addition of parameters) and with an increase in the number of observations, providing a quantitative measure for the balance of complexity and fit; a lower BIC value indicates a better fit (Schwarz, 1978).

The R-squared value is the proportion of the variance in the dependent variable that is predictable from the independent variable, calculated as follows (Healy, 1984).

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

However, it may be possible in linear models without intercept or with nonlinear models for the numerator to be larger than denominator, leading R-squared to be negative (Healy, 1984; Kvålseth, 1985). In addition, the R-squared value tends to increase with additional model parameters, irrespective of the added value of those parameters (Bonate, 2011). Therefore, the adjusted R-

squared is often used for model comparisons. The adjusted R-squared corrects for the addition of parameters (additional degrees of freedom) relative to number of observations, similar to the BIC (Kvålseth, 1985).

$$\bar{R}^2 = 1 - (1 - R^2)\,\frac{n-1}{n-p-1}$$

where p is the total number of parameters in the model, not including a constant term, and n is the number of observations. However, when the number of parameters in the model is negligible compared to the number of observations, the adjusted R-squared approaches R-squared (Kvålseth, 1985). The size of the datasets used in this thesis ranged from 8000 datapoints on individual weekly averages of observations to over 5 million datapoints on each entry of individual birds to the precision feeding system (see for a full description and diagram of this system Zuidhof et al. 2018, US Pat. No. 20180092331). Therefore, the adjusted R-squared was not used in this thesis.

In addition to statistics used for goodness of fit, systemic bias of models can be studied by the linear regression between observed and predicted values (Bonate, 2011). A regression intercept of 0 and a slope of 1 would indicate no bias. If the regression intercept > 0 and slope > 1, the model would systematically underestimate the observed value and if the regression intercept < 0 and slope < 1, the model would systematically overestimate the observed value (Bonate, 2011).

The above described statistics and methods can be used to find models that better approach 'the truth'. It can be argued that the models in this thesis are not 'the truth' or in some cases maybe not even completely represent 'reality'. However, the models will allow to get closer to the understanding of whatsoever things are true.

2.2 The energetic fate of feed

Efficient utilization of feed plays a critical role in the quest for sustainable animal protein production. Feed is associated with 60-70% of the costs of production in broilers (Williams, 1999; Korver et al., 2004; Donohue and Cunningham, 2009). Feed also accounts for 80% of supply chain energy use, 82% of greenhouse gas emissions, 98% of ozone depleting emissions, 96% of acidifying emissions, and 97% of eutrophying emissions of US broiler production (Pelletier, 2008). Therefore, optimizing feed nutrient utilization can reduce environmental footprint of production (Nahm, 2002). Feed provides a source of energy and building blocks to the bird for maintenance, growth, and reproduction (Leeson and Summers, 2001). Characteristics of the environment, the feed, and the animal determine whether the fate of dietary nutrients and energy partitions to waste, maintenance, growth, or reproduction (Leeson and Summers, 2001). Environmental factors include, for example, feed intake, temperature, lighting, disease pressure, or other management related practices. Feed characteristics include, for example, nutrient content, digestibility of the ingredients, feed form, presence of nutritionally active factors, or inclusion of biologically active additives. Animal characteristics include, for instance, age, health status, genotype, or reproductive status.

Poultry feed consists of a blend of several primary ingredients, such as soybean meal, corn or wheat, and canola oil, which provide the protein, carbohydrates, and lipids needed to fulfill the nutrient requirements of the bird (Leeson and Summers, 2001). The aim of the nutritionist is to balance ingredients such that their nutrients are optimally used for product formation at the least costs (Rose, 1997; van Kempen and Simmins, 1997). Crude protein, amino acid, and energy content are the main constraints in least cost feed formulation. Protein is defined as a nutrient and the dietary protein content, including specific amino acids, can directly be measured (Leeson and Summers, 2001). Sufficient quantities of energy are needed for product formation to make optimal use of dietary amino acids (Leeson and Summers, 2001); especially because protein containing ingredients are expensive (Lemme et al., 2004) and nitrogenous waste is polluting to the environment (Nahm, 2002). If amino acids are not used for product formation, they are used as an energy source and nitrogen is excreted (Donaldson et al., 1956; MacLeod, 1997). Therefore, nutritionists use energy to protein ratio as a constraint in diet formulation (Leeson and Summers, 2001). Energy can be used in metabolic processes after energy containing nutrients (protein, lipids, or carbohydrates) are combusted. Combustion processes (glycolysis, beta-oxidation, or the citric

acid cycle) produce adenosine triphosphate (ATP), which provides energy in cellular metabolism (Leeson and Summers, 2001). Because energy is the currency for all metabolic processes including maintenance, growth, and reproduction, studying the partitioning of energy to these processes can provide insight into the efficiency of the animal and the effects of the diet or the environment on energy partitioning. This part of the review will therefore focus on the use of dietary energy in the chicken, or, the energetic fate of feed.

2.2.1 Energy

The laws of thermodynamics state that the total energy of an isolated system is constant (Joule, 1845; Mayer, 1862). This means that energy is not created nor destroyed and energetic input equals energetic output. In addition, the entropy of a system that is not in equilibrium will increase over time, approaching a maximum value at equilibrium (Clausius, 1850). The result of the increase of entropy in a system is that a proportion of energy is transformed into heat, when energy is transformed from one form to the other. If we imagine a chicken as an isolated dynamic system trying to reach equilibrium, all the energy containing nutrients a chicken eats will either 1) be transformed into heat (heat production or maintenance), 2) be transformed into energy containing tissues (retained energy or gain), 3) be transformed into energy containing products (eggs), or 4) leave the chicken through excreta (unmetabolizable energy).

To define energy partitioning to a particular purpose, a framework was created to explain the fate of energy from the feed. In this energy system, the gross energy (GE) of a feed is defined as the total energy released when feed is completely oxidized into carbon dioxide and water (NRC, 1981a, 1994). The GE can be measured using a bomb calorimeter. The digestible energy (DE) is defined as the GE of the feed minus the GE excreted through the feces (NRC, 1981a). As poultry excrete feces together with uric acid, it is easier to measure the metabolizable energy (ME) of a feed, which is defined as the DE of feed minus the energy in uric acid and gaseous products of digestion (NRC, 1981a). In addition, ME is the total energy that can be partitioned to maintenance, growth, and reproduction, therefore also called total useful energy (Zuidhof, 2019). The ME corrected for zero nitrogen retention (MEn) value of a feed (expressed as kcal/kg) is currently the standard energy value in poultry feed formulation (Lopez and Leeson, 2008). The MEn is calculated by subtracting the energy value of the retained nitrogen from the ME value (Hill and Anderson, 1958). Correction for nitrogen retention accommodates the effect of different growth rates between individuals and any age-related effects (Lopez and Leeson, 2008). However, its use

has also been criticized as protein accretion is the norm in poultry production and protein contributes to the total retained energy in the body and products (Zuidhof, 2019). In addition, nitrogen correction penalizes ingredients with high dietary protein content for being used as an energy source, such as soybean meal (Lopez and Leeson, 2008).

ME can be partitioned to retained energy (RE), defined as the energy stored in body tissues or productive output (eggs), and heat production (HP). In addition, overall net energy (NE) is partitioned into NE for maintenance (NE_m) and NE for production (NE_p; NRC, 1981a). The NE_p used by the bird can be calculated as the ME minus total HP, and is equivalent to RE (NRC, 1981a). NE_m is the energy required to maintain an animal in a state where they are neither gaining nor losing weight, at rest in a thermoneutral environment, fasting, and sexually inactive, i.e. sustain its basal metabolic rate (NRC, 1994). In poultry production, this definition is mostly theoretical; the industry aims to not have birds be in their basal metabolic state because birds are kept to produce meat or eggs. When the animal is gaining weight, NE_m is completely lost in HP, consequently, separating NE_m from other sources of HP is difficult and not practically relevant (Zuidhof, 2019). The NE for production (NE_p) of a feed is defined as the net increase in useful product in terms of calories expressed per unit increase in feed consumed (NRC, 1981a), i.e. dietary energy partitioned to gain (retained in body tissues; NE_g) or products (egg production, NE_{egg}). All energy lost as heat and used for maintenance (total HP) is equivalent to the ME for maintenance (ME_m). ME_m includes heat loss from NE_m, digestion, absorption, fermentation, product formation, waste formation and excretion, activity, immune response, and thermoregulation (NRC, 1981a). ME_m or total HP can be calculated as ME minus RE. A summary of the energy framework can be found in Figure 2.1.

The comparative slaughter technique (CST; Fraps and Carlyle, 1939) and indirect calorimetry, also known as respiration calorimetry (Frankenfield, 2010), have been used most commonly to quantify energy partitioning (Birkett and de Lange, 2001). However, both methods assume a fixed value for efficiency of energy retention and estimate the requirements for maintenance, growth, and egg production independently. The CST is terminal, and does not allow for repeated measurement, and indirect calorimetry has limitations in terms of sample size due to high costs of respiration chambers (Romero et al., 2009b). Therefore, mathematical models have been developed to partition energy intake into maintenance, growth, and egg production based empirical feed intake, gain, and egg production data.

2.2.2 Energy partitioning models

Several studies so far have estimated and modeled energy partitioning in chickens. Table 2.1, 2.2, and 2.3 provide a summary of the literature of the estimates of the ME requirements for maintenance, and the ME cost of growth and egg production. Using models to study energy partitioning serves two main purposes: 1) understanding of the ME requirement of maintenance and the ME cost for growth and egg production, 2) testing hypothesis for differences in energy partitioning between treatments. The final goal is to optimize energy partitioning to productive purposes (lean tissue or eggs) by finding ways to reduce the ME_m or balance growth and egg production. The fundamental basis of the models used for energy partitioning is that daily ME intake (MEI; kcal/d) is assumed to be the sum of the ME used for maintenance (ME_m), for gain, and for egg production, therefore:

$$\text{MEI} = [\text{maintenance}] + [\text{gain}] + [\text{egg production}]$$

ME_m requirements were determined to be proportional to the body weight and body surface area (Kleiber, 1947). Kleiber observed that, for the majority of animals, their metabolic rate scales to the power 0.75 of their mass, called Kleiber's law (Kleiber, 1947). However, several powers of body weight have been used to estimate body surface area from body weight. Brody (1945) concluded that a power of 0.67 was fitting better for birds. The ME for gain would be correlated with the average daily gain, so traditionally, average daily gain has been used as an estimator for ME for gain (NRC, 1981b). Similarly, egg mass is correlated to the daily energy partitioning to egg production and therefore incorporated in the equation. The traditionally used energy partitioning model was defined as follows:

$$\text{MEI} = a \times \text{BW}^{0.75} + b \times \text{ADG} + c \times \text{EM}$$

Where BW = BW (kg), ADG = gain (g/d), and EM = egg mass produced (g/d) (Byerly, 1941; Valencia et al., 1980; Byerly et al., 1980; Sakomura et al., 2003; Pishnamazi et al., 2008; Reyes et al., 2011, 2012). EM has also been replaced by egg production in number of eggs in some cases. It was argued that the two major constraints underlying the relationship between BW and ME_m requirements (surface-area limits on resource and waste exchange processes and mass and volume limits on power production) would be able to explain some of the variation in ME_m requirements (Glazier, 2005). In addition, Romero et al. (2009b) posed that relaxing the assumption of a fixed exponent of metabolic BW may be a critical to improve fit and reduce bias in energy partitioning

models (Romero et al., 2009b). To mitigate the issue on the fixed power value the following model was proposed:

$$\mathrm{MEI} = a \times \mathrm{BW}^{b} + c \times \mathrm{ADG} + d \times \mathrm{EM}$$

Where b has previously been estimated as 0.54 (Romero et al., 2009a), 0.67 (Zuidhof et al., 2017), 0.68 (Hadinia et al., 2018), and 0.84 (Pishnamazi et al., 2015) for broiler breeders. The variation in the estimation of b may originate from differences in age (pullet or mature birds), differences in feed allocation between studies, housing type, environmental circumstances, or potentially interactions between those factors.

One of the limitations of the above model is that the ME cost for gain (c) is linear. Weight gain is composed of tissues of varying energetic density, e.g. fat (9.1 kcal/g dry matter) or protein (5.5 kcal/g dry matter; Atwater, 1900). In addition, lean tissues also contain around 75% of water (Qiao et al., 2001), which has no retained caloric value (0 kcal/g). Young animals deposit more protein than fat, and mature animals tend to deposit more fat than protein (Nürnberg et al., 1998). Therefore, the ME cost for gain would depend on age. To illustrate this, Table 2.2 provides an overview of reported ME requirements for gain of mature and pullet broiler breeders as found in the literature based on energy partitioning models. Average ME costs for gain were 3.41 ± 1.45 kcal/g for mature broiler breeders, 1.92 ± 1.09 kcal/g for broiler breeder pullets. Hence, this supports the hypothesis that pullets would have a lower ME cost for gain compared to mature birds, as pullets deposit more protein than fat. As time or age are correlated with BW, BW can be used for adjustments for allometric (proportional change) differences instead of age. Allometric composition differences in gain change the ME cost for gain over the growth curve. Birds with higher BW (older birds) would deposit more fat than protein, and therefore require more energy per unit of gain (Romero et al., 2009b). The following model was proposed, including exponents to gain and the interaction between BW (BW^{e}) and gain (Romero et al., 2009b), which allow for a higher energy requirement per unit of gain in heavier birds, an a non-linear gain in a Cobb-Douglas functional form (Griffin et al., 1987):

$$\mathrm{MEI} = a \times \mathrm{BW}^{b} + \mathrm{c} \times \mathrm{ADG}^{d} \times \mathrm{BW}^{e} + f \times \mathrm{EM}$$

When MEI is lower than the combined $\mathrm{ME_m}$ costs and ME cost for egg production, gain can also be negative, i.e. energy from body reserves is released for $\mathrm{ME_m}$ requirements or egg production. This resulted in the following equation (Romero et al., 2009b):

$$\mathrm{MEI} = a \times \mathrm{BW}^{b} + c \times \mathrm{ADG}_{\mathrm{P}}^{d} \times \mathrm{BW}^{e} + f \times \mathrm{ADG}_{\mathrm{N}}^{g} \times \mathrm{BW}^{h} + i \times \mathrm{EM}$$

where ADG_P = positive gain (g/d) and ADG_N = weight loss (g/d). When $ADG_P > 0$ then $ADG_N = 0$, and vice versa, when $ADG_N > 0$ then $ADG_P = 0$. As model fitting programs compute the derivatives of these functions when calculating the likelihood functions and the log(0) is undefined, these models would not converge. A method to resolve this issue was to replace in the dataset zeros by a small value and correct ADG_P or ADG_N by this value. For example, if $ADG_P = 50$ and $ADG_N = 0$ then $ADG_P = 50.0001$ and $ADG_N = 0.0001$. Mathematically this is correct, however, it can be argued that manipulating datasets is not preferred as technically weight loss and weight gain are not measured simultaneously. Yet, from a biological standpoint, protein turnover technically represents a simultaneous gain and loss at all times (Muramatsu and Okumura, 1985). Similar to the ME cost for gain, the cost for EM (egg mass) may differ at different BW. During the first stage of production, when EM is increasing, eggs increase in gross energy content (Chwalibog, 1992). This could be associated with a greater deposition of yolk. Romero et al. (2009b) found a strong positive correlation (r = 0.85) between EM production and percentage of yolk in the egg in broiler breeders from wk 30 to wk 60. In addition, it was hypothesized that as a result of a greater availability of liver lipids, there could be a greater efficiency for energy retention in eggs in birds with greater BW (Romero et al., 2009b). This led to the following equation (Romero et al., 2009b):

$$\mathrm{MEI} = a \times \mathrm{BW}^{b} + c \times \mathrm{ADG}_{\mathrm{P}}^{d} \times \mathrm{BW}^{e} + f \times \mathrm{ADG}_{\mathrm{N}}^{g} \times \mathrm{BW}^{h} + i \times \mathrm{EM}^{j}\mathrm{BW}^{k}$$

Whether or not adjusted for level of EM production and/or BW, ME costs for EM production have been consistently estimated between 1.79 and 3.1 kcal/g egg for both broiler breeders and laying hens (Table 2.3).

In the past, fitting more complex models like the above was limited by computational power and the time for complex models to converge. This issue has been resolved to a large degree with advancements in computing science. However, the available information on BW, gain, weight loss, or egg mass may still be limited (e.g. not many individual birds lose weight over the experiment). When insufficient variation or degrees of freedom are available, or autocorrelation is present, models may still not converge. In addition, increasing complexity of the model in one area will sometimes make it necessary to simplify other areas. For example, adding a random term to ME_m requirement may entail elimination of the parameter for weight loss. Under those circumstances it is a matter of balancing simplicity with the comprehensive value of a variable (Domingos, 1999), as earlier discussed in the section on modeling.

2.2.3 Maintenance

A large proportion of ME intake in chickens is partitioned to maintenance (Latshaw and Moritz, 2009). In broilers between 1.1 and 2.2 kg, ME_m requirement was approximately 50 to 100% of ME intake, where increased ME intake decreased the proportion of ME released as heat (Latshaw and Moritz, 2009). In mature chickens, ME_m requirement was estimated 59.8% and 64.9% of ME consumption for laying hens (Reid et al., 1978) and broiler breeders (Rabello et al., 2006), respectively. Table 2.1 provides an overview of estimates for ME_m requirements found in the literature, focusing on broiler breeders and laying hens. Overall average estimates for ME_m requirements standardized to 1.5 kg were 116.8 ± 20.2 kcal/kg$^{0.67}$ for mature broiler breeders, 125.3 ± 18.6 kcal/kg$^{0.67}$ for broiler breeder pullets, 91.3 ± 10.9 kcal/kg$^{0.67}$ for mature laying hens, and 105.9 ± 51.9 kcal/kg$^{0.67}$ for pullet laying hens. Hence, mature birds have a lower ME_m requirement compared to pullets. The difference between pullets and mature birds may stem from a difference in ME utilization efficiency for production purposes. If the efficiency of ME utilization for gain or egg production is low, the proportion of ME not used for production is part of ME_m and released as heat. The efficiency of ME utilization for gain was lower than the efficiency of ME utilization for egg production in adult broiler breeders (47% vs 64%, respectively; Rabello et al., 2006), but they were similar in laying hens (65% and 62%, respectively; Sakomura, 2004). The difference between laying hens and broiler breeders may originate from an increased basal metabolic rate in broiler breeders due to increased proportional lean tissue content of the body. Firstly, broiler breeders have been bred for fast and lean growth (Renema et al., 2007), while laying hens have been bred for egg production, including early onset of lay (Bain et al., 2016), which requires early lipid deposition (Kwakkel et al., 1991). Second, broiler breeders are severely feed restricted (Decuypere et al., 2010), resulting in extremely lean birds (Zuidhof, 2018). The basal metabolic rate is higher for lean tissue compared to fat tissue (Scott and Evans, 1992), therefore, the basal metabolic rate for broiler breeders may be higher compared to laying hens due to increased lean or decreased fat body content. It is hypothesized that the relative difference in lean tissue would predominantly cause the difference in ME_m, as the heat associated with whole-body protein turnover was estimated to range between 14 and 21% of the basal metabolic rate in broilers at d 14 (Muramatsu et al., 1987). Following the same reasoning, pullets could also have a higher basal metabolic rate compared to mature birds, because of the relative higher proportion of lean tissue (Kwakkel et al., 1991; Lesuisse et al., 2017). The most

common feeding practice in broiler breeders is once-a-day or skip-a-day feeding, whereas for laying hens birds are fed *ad libitum* or close to *ad libitum*. As a consequence, birds to go through a feeding and fasting cycle, which requires nutrients to be stored and released, respectively (Zuidhof, 2018). Storing and releasing nutrients is associated with a metabolic cost (Hadinia et al., 2018), which could also explain the higher ME_m requirements in broiler breeders compared to laying hens.

Although the previously mentioned averages are calculated from literature varying in breed and housing circumstances, the differences pose interesting questions. How much individual variation exists between birds in their prioritization of energy partitioning between maintenance, gain, and reproduction? Do birds experience a metabolic shift in prioritization of energy partitioning or ME utilization when sexual maturity is reached, that would allow them to allocate more ME away from ME_m requirement or gain to egg production? It is generally assumed that mature birds would prioritize egg production over gain once they have reached their mature BW; *ad libitum* fed broiler breeders indeed only marginally gain weight during the mature phase (Yu et al., 1992c). Under feed restricted circumstances, it is recommended that broiler breeders stay in positive energy balance throughout the laying phase to sustain egg production (Aviagen, 2016). This poses the question, what is the optimal balance between growth and reproduction? How does individual variation in ME_m requirements affect this balance? Hens with a lower ME_m requirement partitioned more energy toward chick production than hens with high ME_m requirements (Romero et al., 2009a), therefore, a reduced ME_m requirement during the mature phase increased ME output towards reproduction. This indicates that breeding for reduced ME_m requirements may improve the prioritization of ME partitioning to reproduction.

In addition to differences in ME_m requirements between individuals, the environment of the animal (e.g. temperature and housing conditions) can also influence the ME_m requirement of a bird. Variations on the equations discussed in the previous section allow for adjustment for differences in environmental temperature (NRC, 1981b; Sakomura, 2004; Pishnamazi et al., 2015). However, as temperature is not the main focus of this thesis, these will not be discussed. With regards to individual ME_m differences, Romero et al. (2009b) proposed an adjustment to the above models adding a random variation component to the ME_m requirements:

$$\mathrm{MEI} = (a + u) \times \mathrm{BW}^{b} + c \times \mathrm{ADG} + d \times \mathrm{EM}$$

Individual hen specific random term $u \sim N(0,V_u)$ was included to separate individual variation linked to maintenance from other sources of variation. The SD of ME_m requirements ($\sqrt{V_u}$) ranged between 4.6 kcal/kg$^{0.67}$ to 23.6 kcal/kg$^{0.67}$ for 1.5 kg birds in previously estimated ME_m requirements for pullet and mature broiler breeders (Romero et al., 2009a; b; Pishnamazi et al., 2015; Hadinia et al., 2018), and broilers (Zuidhof et al., 2014). Housing and feed allocation were hypothesized to be the main differences between studies, where group housing compared to individual housing and larger differences in feed allocation result in larger individual variation in ME_m requirements. Birds in groups can display individual differences in activity levels and increased feed intake increases the heat increment of feeding (NRC, 1994). The between-bird variation in ME_m requirements would allow for selecting the most efficient individuals. Including the bird-specific random term also allows for the calculation of residual ME_m requirement, also referred to as residual HP (RHP). RHP is the residual of the linear relationship between ME_m requirement and ME intake (Romero et al., 2009a; Hadinia et al., 2018). The RHP measures energetic efficiency without being confounded by feed intake (including dietary thermogenesis), BW gain, or egg production, i.e. corrects for bias against high producing animals. RHP was higher for hens fed a low energy diet compared to hens fed a high ME diet (Pishnamazi et al., 2015), but did not differ between individually fed cage housed and group fed cage housed broiler breeders (Romero et al., 2009a) or conventionally fed and precision fed group housed broiler breeders (Hadinia et al., 2018). This may suggest that RHP could indeed be independent of feeding level and feeding management. RHP was higher for female broilers (5.44 ± 1.63 kcal/kg$^{0.6}$/d) compared to male broilers (-8.34 ± 1.63 kcal/kg$^{0.6}$/d; Romero et al., 2011). This was hypothesized to be the result of allometric growth differences between male and female broilers, as the maturation rate of breast muscle is higher in females, which increases their maintenance requirements (van der Klein et al., 2017). The SD of RHP was estimated at 4.1 kcal/kg$^{0.54}$ and did not differ between individually fed cage housed and group fed cage housed broiler breeders (Romero et al., 2009a).

2.2.4 Consequences of controlling energy balance

In healthy birds, energy intake is regulated through complex endocrinological processes and interactions in the hypothalamus (Richards and Proszkowiec-Weglarz, 2007). Broiler breeders are unable to control their feed intake and therefore lack the ability to balance their energy partitioning to maintenance, growth, and egg production in favour of egg production during the mature phase (Richards et al., 2010). Therefore, the current practice is to control energy or feed

intake by controlling BW to steer their energy balance towards egg production. Both energy abundance and energy restriction lead to metabolic (Mitchell, 1962) and behavioural adaptations (Dixon et al., 2014). It is imperative to note that the current (and increasing) feed restriction in broiler breeders is a big (and growing) welfare concern (Mench, 2002; Jong et al., 2002; Decuypere et al., 2010; Dixon et al., 2014). Yet, a refined definition of the 'level of restriction' for broiler breeders can be hard to find. The level of feed restriction is most often defined as a proportion of what would be consumed under *ad libitum* fed circumstances. For example, a 20% feed restriction would be equivalent to a feed allowance of 80% of *ad libitum*. However, as earlier mentioned, broiler breeders overconsume energy under *ad libitum* circumstances, beyond their requirements for maintenance, growth, and reproduction (Richards et al., 2010) and are metabolically unhealthy if they do so (Yu et al., 1992a; Chen et al., 2006). For example, *ad libitum* broiler breeders display erratic oviposition and defective egg syndrome (Yu et al., 1992a) and lipotoxicity-like symptoms (Chen et al., 2006), due to the accumulation of excess triacylglycerol and fatty acids in nonadipocytes resulting in altered intracellular signaling, cellular dysfunction, and cell death (Unger, 2002). Potentially a better way to describe energy restriction is to relate restriction to ME_m requirement, i.e. associate restriction as ratio of intake vs metabolic BW. Even so, at the same level of feed or energy allowance, individuals will receive varying levels of restriction relative to intrinsic reference parameters, such as their genetic growth potential (mature BW) or individual requirements for ME_m.

In broilers, energy restriction has been used to steer the energy balance from ME_m towards gain. Energy restriction improved the efficiency of ME utilization for gain, as lower overall ME_m requirements and compensatory growth improved feed efficiency (Schwean-Lardner et al., 2012) and energy restriction reduced the basal metabolic rate in animals (Mitchell, 1962). Modest short term feed restriction can also alter allometric growth in broilers (van der Klein et al., 2017). Feeding 70% of *ad libitum* in wk 2 reduced fat pad weight at d 42, but feed restriction in wk 3 reduced breast muscle weight (van der Klein et al., 2017). However, long term energy restriction as used in broiler breeder pullets results in important alterations in the maturation of the reproductive tract (Bruggeman et al., 1999). Feed restriction applied from wk 7 to 15 followed by either *ad libitum* feeding or continued feed restriction improved egg production compared to treatments fed *ad libitum* between wk 7 to 15, independent from the feeding regime from wk 0 to wk 7 (Bruggeman et al., 1999). This highlights that the degree and timing energy restriction will

determine the direction of the energy balance between maintenance, (the composition of) gain, and reproduction.

The industry strives to direct ME utilization towards production purposes and to minimize the ME_m requirements. However, components of the ME_m requirements are essential to the holistic functioning of the bird and therefore production responses, such as immune response and disease resistance. Changes in the energy balance can lead to changes in the innate and adaptive immune system in both broilers and layers (Klasing, 1988; Hangalapura et al., 2005; Orso et al., 2019). Moderate levels of feed or energy restriction are thought to enhance immune system functionality (Jang et al., 2009), but severe levels can diminish immune responses (Klasing, 2007). For example, feed allowance of 60% or 80% of *ad libitum* for 6 wk in rearing pullets resulted in lower natural antibody levels against lipoteichoic acid compared to *ad libitum* fed birds, but not in specific antibody responses to keyhole limpet hemocyanin (Hangalapura et al., 2005). In addition, it was suggested that birds under prolonged feed restricted conditions would not be able to maintain T-cell proliferative capacity (Hangalapura et al., 2005). Therefore, reducing the energy partitioning to ME_m may also reduce energy partitioning to the (responsiveness of) the immune system and could potentially lead to reduced disease resistance, especially in severely restricted broiler breeders.

2.3 Reproduction[1]

2.3.1 Introduction

The broiler industry faces challenges in its ability to optimize efficiency of hatching egg production. Even though intensive selection pressure for meat yield has resulted in tremendous improvement in growth rate, feed efficiency, and body composition, it has also significantly altered the reproductive efficiency of broiler breeders (Zuidhof et al., 2007). Under current management practices, broiler breeder body weight needs to be controlled through feed restriction to maintain optimal reproductive performance (Renema et al., 2007; Zuidhof et al., 2014). Decuypere et al. (2010) framed this issue as a paradox between acceptable reproduction and health versus impaired welfare. Understanding the underlying mechanisms responsible for the limitations of reproduction might provide solutions for the current problems and potentially identify new traits for genetic selection. The study of the diurnal and seasonal endocrine dynamics in egg production became prominent during the 1970s, when methods became available to analyze blood, tissue samples, and cell cultures to detect and quantify hormones profiles. Since then, a solid foundational understanding of the endocrinology of egg production has been established (Wilson and Sharp, 1975; Williams and Sharp, 1978; Robinson and Etches, 1986; Robinson et al., 1988; Dunn and Sharp, 1990; Sharp, 1993). However, the most proximate factors on the transcriptomic level underlying the control the initiation of sexual maturation, ovarian cyclicity, and oviposition have yet to be unraveled. This review presents the gaps in current knowledge of the deficiencies in diurnal and seasonal dynamics in egg production in broiler breeders.

2.3.1.1 General overview and regulation of the avian reproductive axis

The brain and the ovary are the two major organs regulating reproduction in birds. Endocrine signaling from these organs initiates reproduction, the dynamics of ovulation, egg formation, and oviposition in response to internal and environmental cues. In short, deep brain photoreceptors receive the most important environmental cue, the length of the photoperiod, to initiate sexual maturation and regulate daily egg production (Dunn and Sharp, 1990). Gonadotropin releasing hormone (GnRH) and gonadotropin inhibitory hormone (GnIH) are produced by and released from the hypothalamus in response to the photoperiod and, respectively,

[1] A - by the editor in chief significantly altered - version of this section of the literature review has been accepted for publication under the title *"Diurnal and seasonal dynamics affecting egg production in meat chickens: a review of mechanisms associated with reproductive dysregulation"* by S.A.S. van der Klein, M.J. Zuidhof, and G.Y. Bédécarrats in Animal Reproduction Science.

stimulate and inhibit the production and pulsatile release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary (Kirby et al., 2005; Bédécarrats, 2015). The ovary produces estradiol-17β (E2) and progesterone in response to circulatory FSH and LH (Robinson and Etches, 1986), and E2 and progesterone feed-back on the hypothalamus to regulate GnRH release (Etches, 1990; Li et al., 1994). This system is called the hypothalamic-pituitary-gonadal (HPG) axis (schematic overview in Figure 2.3).

Deep brain photoreceptors play a key role in the translation of the photoperiodic signal into a neuroendocrine signal, i.e. the production and release of GnRH (Saldanha et al., 2001). Of all the subtypes of GnRH, GnRH-I is the form released in the median eminence to modulate the rate of synthesis and secretion of LH and FSH (Mikami et al., 1988; Sharp et al., 1990; Kuenzel and Blähser, 1991; van Gils et al., 1993). Two photopigments have been identified to be involved in photoperiodic responses of the brain in the reproductive axis: vertebrate ancient (VA) opsin and opsin 5 (also referred to as neuropsin or OPN5; Nakane et al., 2010; García-Fernández et al., 2015). The VA opsin was co-localized with GnRH-I in neurons in the paraventricular organ (PVO) of the posterior hypothalamus (García-Fernández et al., 2015). The VA opsin was also co-expressed within GnRH-I neurons, which indicates that GnRH-I producing neurons could be endogenously photosensitive (García-Fernández et al., 2015). It is not yet confirmed that a mechanistic link exists between VA opsin and GnRH production or secretion. However, Opsin 5 has been mechanistically linked to the release of GnRH-I in quail (Nakane et al., 2010). Long photoperiods (> 12 h of light) induce an increase in thyroid stimulating hormone β (*TSH*β) expression in the pars tuberalis, which results in an increase in thyroid stimulating hormone (TSH). TSH then induces the expression of type 2 deiodinase (Dio2), that converts thyroxine to bioactive triiodothyronine (T3; Nakane et al., 2010; Nakane and Yoshimura, 2010; Ikegami and Yoshimura, 2012). Finally, T3 induces GnRH secretion (Yasuo et al., 2005). During short days (< 8 h of light), Dio2 mRNA levels are down regulated and type 3 deiodinase (Dio3) mRNA levels are upregulated, which inactivates T3 and leads to a reduction in GnRH secretion. In chickens, photostimulation (the change from short to long photoperiods) rapidly enhances expression of *TSH*β within the pars tuberalis of the pituitary gland in both the Red Jungle Fowl and laying type birds (Ono et al., 2009; Dunn et al., 2017). Also, *in vivo* inhibition of Opsin 5 by siRNA in neurons of the PVO resulted in the reduction of *TSH*β expression (Nakane et al., 2014) and the ratio of Dio2/Dio3 expression increases after photostimulation (Dunn et al., 2017). Therefore, Opsin 5 makes a likely candidate to be involved

in translating the photoperiodic signal into a neuroendocrinological signal. Yet, the VA opsin and opsin 5 pathways do not have to be mutually exclusive. In a complementary fashion, the VA opsin pathway could control the synthesis of GnRH-I while the opsin 5 pathway could control GnRH-I release.

An inhibitory hypothalamic factor was first discovered in quail and named GnIH (Tsutsui et al., 2000). The GnIH peptide directly inhibits hypothalamic GnRH-I and GnRH-II release (Satake et al., 2001; Bentley et al., 2003; Osugi et al., 2004; Ubuka and Bentley, 2011) and the synthesis and release of pituitary gonadotropins (Ciccone et al., 2004; Ubuka et al., 2006). The release of GnIH is stimulated by melatonin and, therefore, longer scotoperiods increase GnIH signaling (Bédécarrats et al., 2016). Some have argued that GnIH plays a direct role at the ovarian level as well, affecting follicular recruitment, sexual maturation, and steroid production (Maddineni et al., 2008b; a; Tsutsui et al., 2010). *In vitro* studies concluded that E2 and progesterone downregulate GnIH receptor (*GPR74*) expression in thecal and granulosa cells. However, others stress that evidence is still needed to support GnIH expression at the ovary and argue that another GnIH receptor (*GPR147*), which has a much higher affinity for GnIH, is not expressed in ovarian tissues (Ubuka et al., 2013). The area of research around GnIH is relatively new, but it is important to understand whether the deep brain photoreceptors directly or indirectly influence GnIH signaling and whether GnIH has a potential direct role in the ovary.

2.3.2 Diurnal dynamics

The diurnal dynamics of ovulation and oviposition are controlled through endocrine pathways that are under the influence of both environmental and internal factors. The first few paragraphs of this section will describe the current knowledge on rhythmicity of ovulation and oviposition in relation to environmental cues, the second section will further explore the endocrinological processes underlying ovulation, and finally the underlying mechanisms of dysregulation of the ovary in the broiler breeder will be discussed.

2.3.2.1 Photoperiod entrained rhythmicity

Chickens are indeterminate layers, which means that they do not constrain their egg-laying sequence length to a fixed number of eggs (Etches, 1990). Laying sequences in chickens can be defined as coupled, when the current and preceding sequence are separated by a single pause day, or uncoupled, when separated by two or more pause days (Fraps, 1965). Three timely separated events define the egg laying sequence (successive lay of more than one egg). The first event in a

sequence is the surge in LH release, second is the occurrence of ovulation, and third is oviposition (Figure 2.3). The most important hormones related to rhythmicity of the reproductive axis are progesterone, released by the mature follicle, GnRH, released by the hypothalamus, and LH, released by the pituitary. A positive feedback loop between progesterone, GnRH, and LH will result in the LH surge and ultimately ovulation. The timing of the LH surge, oviposition, and ovulation depends on the preceding event, follicle and egg maturation, and environmental cues. The most important environmental cue is the photoperiod, where the transition from light to dark (dusk) entrains the timing of ovarian cycles (Etches, 1990). Under normal 24 h days with a photoperiod of 14 h light and 10 h dark, the LH surge can only occur within an 8 h "open period" (Fraps, 1954; Etches, 1990). The time of maturation of the next follicle, i.e. the ability of the next follicle to respond to the progesterone-GnRH-LH positive feedback loop, needs to coincide with this "open period". Approximately 4 h following the LH surge, ovulation occurs. Ovulation is followed by oviposition between 24 to 28 h later. Ovulation of the next follicle usually occurs within 15 to 45 minutes after oviposition, if the LH surge coincided with the open period. Little attention has been paid to the neuroendocrine mechanisms behind the LH "open period", and the links between the LH "open period", the initiation of the scotophase (darkness), and the deep brain photoreceptors.

Under normal 24 h days, oviposition occurs within a 10 h period following the initiation of the photophase. Conversely, under constant light or constant dark photo regimes, oviposition occurs at any time during the day, however, the times of oviposition are not equally dispersed over the day (Bhatti, 1987). Etches (1990) interpreted this phenomenon as evidence that some hens might use other environmental cues, such as temperature, sound, or feeding cycle to set the periodicity of oviposition to a restricted 8 h period during the day. Backhouse and Gous (2006) concluded from a literature review that delaying feeding time during the photoperiod affected time of oviposition in broiler breeders, and results from more recent research indicated that afternoon feeding decreased egg production compared to morning feeding (Londero et al., 2015). However, the biological factors behind the interaction between feeding time and the reproductive system are still unclear.

The study of the rhythmicity and circadian entrainment of egg production has focused primarily on laying type breeds (Etches and Schoch, 1984). Full-fed broiler breeders do not seem to follow the models describing ovulatory cycles developed for feed restricted broiler breeder hens

or laying hens (Álvarez and Hocking, 2007), which indicates that their reproductive cycle is distorted (Hocking et al., 1987). Although egg production patterns of feed restricted breeder hens are characterized by shorter laying sequences compared to laying-type hens (Gumulka et al., 2010), several authors suggested that feed restricted broiler breeders follow the same models describing rhythmicity and circadian entrainment of egg production as laying hens (Gous and Nonis, 2010; Gumulka et al., 2010; Ferreira et al., 2016). Recent findings showed that a restricted-fed Cobb parent line had a prime sequence length (longest sequence length within a cycle) of 22.38 ± 13.5 d, an average sequence length of 3.73 ± 1.32 d, and an average pause length of 1.25 ± 0.28 d over 51.28 ± 12.49 sequences and 50.28 ± 12.49 pause days during a production cycle from 24 to 60 weeks of age (Ferreira et al., 2016). In comparison, a Hy-Line Brown laying hen line had a longer prime sequence length of 83.44 ± 41.9 d, a longer average sequence length of 23.16 ± 11.7 d, and a similar pause day length of 1.48 ± 0.9 d over 10.68 ± 7.4 sequences and 9.68 ± 7.4 pause days to week 44 of the egg laying period (Johnston and Gous, 2007). Genetic selection for increased egg production in laying hens may have largely eliminated pause days and increased the sequence length, but potentially also created differences in the (hypothalamic) clock dependent mechanisms. The (neuro)endocrine origin of the differences between laying hens and broiler breeders affecting diurnal egg production patterns could give insight into the reproductive issues of (full-fed) broiler breeder hens.

2.3.2.2 Follicular maturation and recruitment

The mechanisms behind follicular maturation and recruitment, including the steroidogenic capacity of the different follicles and cell-types are important, as these mechanisms may underlie the basis for understanding the issues of double hierarchies (multiple follicles at the same maturity), double yolked eggs, and erratic oviposition in broiler breeders. In the healthy mature chicken ovary, three categories of follicles can be identified; small primordial follicles (~1-5 mm), pre-recruitment follicles (6-8 mm), and pre-ovulatory follicles (>8 mm; Figure 2.4a). Within the pool of pre-ovulatory follicles, follicles can be classified in relation to their proximity to ovulation as F1 (largest and next for ovulation) through F5-F7 (smallest). In contrast, in full-fed broiler breeders, a double hierarchy can occur (Figure 2.4b), where two hierarchical follicles are identical in size. Although the size indicates the proximity to ovulation, it is only the responsiveness to the progesterone-GnRH-LH positive feedback and the ability to produce progesterone in large quantities that determines whether a follicle is mature. The slow growing pre-recruitment follicles

(formerly known as pre-hierarchical follicles) were recently determined to also have a hierarchical order related to their proximity to recruitment (Ghanem and Johnson, 2019a). In addition to follicles taken up in the hierarchy for ovulation, atretic follicles and post-ovulatory follicles can also be present in the ovary. Atretic follicles originate from pre-recruitment follicles, but seldom from pre-ovulatory follicles (Etches, 1990). The granulosa layer and theca layers surrounding the follicles produce steroids in response to LH and FSH. In the theca interna of primordial and pre-recruitment follicles androstenedione is predominantly produced from pregnenolone through the Δ5-steroidogenic pathway, which is then converted into E2 in the theca externa layers and released into the blood. In pre-ovulatory follicles less advanced into in the hierarchy (F2-F5), the theca interna follicles utilize the Δ4-steroidogenic pathway instead of the Δ5-steroidogenic pathway due to a decreased sensitivity to LH of the theca interna (Robinson and Etches, 1986), hence, androstenedione is predominantly produced from progesterone (Lee et al., 1998). The granulosa cells of pre-ovulatory follicles have a greater sensitivity to LH and lesser sensitivity to FSH compared to pre-recruitment follicles, which gradually advances with proximity to ovulation (Johnson, 1990). This coincides with a decrease in P450 aromatase and E2 production by the theca externa. The exception to this pattern of steroidogenesis is the F1 follicle, where the Δ5-pathway appears to be present, but the theca interna does not produce any androstenedione. It is thought that the lack of dihydroepiandrosterone precursor results in a non-functional Δ5-steroidogenic pathway in F1 theca cells (Lee et al., 1998). In addition, there is a marked increase in progesterone production in the granulosa layer of the F1 follicle. This progesterone surge is inducing the LH surge resulting in ovulation of the F1 follicle (Etches, 1990). Table 2.4 provides an overview of the steroids produced by the follicles in their respective maturation stage and their steroidogenic capacity. It has been suggested that small pre-ovulatory follicles influence the progesterone production of larger follicles through their testosterone production (Rangel et al., 2014), therefore, the interactions between follicles and as such ovary as a system seems to be responsible for the ovulation of the F1 follicle by coordinating the activity of follicles within the hierarchy, and possibly follicle recruitment processes as well.

Two follicle selection processes take place in the ovary of the mature hen. The first is the selection of follicles into the pre-recruitment pool and the second is the selection of the follicles into the pre-ovulatory pool. In this process, granulosa cells of the primordial follicles acquire the ability to respond to FSH. A working model of the cellular mechanisms behind desensitization of

the FSH receptor in granulosa cells of pre-recruitment follicles has been previously reviewed by Johnson and Woods (Johnson and Woods, 2009), which has been further supported by several new studies (Kim, 2013; Johnson and Lee, 2016; Kim and Johnson, 2018a). Interestingly, the proposed mechanisms have also been linked to the arrhythmicity of peripheral clock genes through vasoactive intestinal peptide receptors (Kim and Johnson, 2016, 2018b), supporting the hypothesis of an internal ovarian clock mechanism related to cyclic recruitment of follicles. Previously it was assumed that recruitment of follicles and the timing of ovulation, including the LH "open period", were controlled solely by photoperiod and a clock-dependent mechanism within the neuroendocrine system. The rhythmicity of ovulation could also be in part controlled by clock-dependent mechanisms within the follicles. In both theca and granulosa cells, LH binding regulates steroidogenic acute regulatory protein expression (*StAR*), which mediates cholesterol uptake into the mitochondria. In quail, expression of *StAR* mRNA levels in the F1 follicle changes over the course of 24 h, coinciding with changes in clock gene *Per2* expression (Nakao et al., 2007). It has been suggested that this mechanism is similar in chicken (Ono et al., 2009). The follicular recruitment and ovulation related intra-ovarian clock mechanisms may also work independently, because the cyclic recruitment of follicles occurs independently of ovulation (Ghanem and Johnson, 2019b). It is still unclear how the neuroendocrine and ovarian clock system(s) interact or whether these systems differ between layer and broiler breeds. One possibility is that this interaction lies in the direct innervation of the theca interna cells (Gilbert, 1969; Bahr et al., 1986; Ebeid et al., 2008; Johnson and Woods, 2011), in addition to circulatory endocrines. Although the innervation of the ovary, oviduct and, shell gland by adrenergic, cholinergic, and catecholaminergic neurons has been identified (Sturkie and Freedman, 1962; Gilbert, 1969; Unsicker et al., 1983), the functionality of this innervation and the effects of the neurochemicals have not been evaluated with modern research techniques. For example, the presence of neuroendocrine cells was identified in the chicken ovary only more recently (Hofmann et al., 2013).

After follicle selection, the acquired cell signaling initiates several processes critical to the final growth and differentiation of pre-ovulatory follicles, including the rapid uptake of yolk lipids (Johnson and Woods, 2009; Johnson, 2015). The variety of growth factors required for development of follicles to progress through the hierarchy have been reviewed by Onagbesan et al. (2009). Walzem and Chen (2014) also reviewed the regulation of the uptake of yolk lipids in

relation to metabolic status. Subsequently, more hormones involved in follicle stimulation and maturation have been discovered. For example, growth hormone was found to be an important stimulator of E2 production in pre-hierarchical follicles (Hrabia et al., 2012) and prostaglandin, E2, and insulin-like growth factor I (IGF-I) interact to enhance proliferation of theca externa cells (Jia et al., 2013). Many new regulatory interactions have been identified between hormones within follicle steroidogenesis and maturation, however, the proximate factors for follicle selection and maturation and the complete integration within the HPG-axis have not yet been elucidated.

2.3.2.3 Reproductive dysregulation in mature broiler breeders

Various differences have been identified between feed restricted and full-fed birds in terms of the endocrine characteristics on systemic and cellular levels (Yu et al., 1992b; Bruggeman et al., 1997, 1999). However, the factors involved in the link between nutrient intake in broiler breeders and its direct effects on the ovary and follicle selection are not fully elucidated and appear to be very complex (Renema et al., 2007). The direct endocrine and metabolic effects of feed restrictive practices related to the hypothalamic melanocortin system have been previously reviewed (Richards et al., 2010). The interaction between the hypothalamic melanocortin system and the HPG axis was a starting point in finding answers for the dysregulation of the reproductive system in broiler breeders. For example, neuropeptide Y (NPY) has been previously suggested to act at the level of the median eminence to stimulate the release of GnRH-I (Contijoch et al., 1993). Stimulation of NPY neurons in the hypothalamus increases feed intake and energy storage and fasting (feed restriction) increases NPY expression (Richards et al., 2010). However, differences in hypothalamic NPY expression patterns have not always been consistent between genetic lines or between levels of feed restriction (Boswell et al., 1999; Byerly et al., 2009; Yuan et al., 2009). Even so, increased brain NPY has been associated with the onset of puberty in male broilers (Fraley and Kuenzel, 1993). Single nucleotide polymorphisms within the NPY gene were associated with total egg production (Wu et al., 2007) and a dominance effect of NPY was found for age at first egg (Dunn et al., 2004). Furthermore, in adult doves, fluctuations in NPY mRNA expression in the mediobasal hypothalamus were correlated with fluctuations in energy state during the breeding cycle (Ramakrishnan et al., 2007). Overall, the literature directs towards a further investigation of the link between the hypothalamic melanocortin system and the HPG-axis, potentially including Agouti-related peptide, proopiomelanocortin, and leptin (Boswell and Dunn, 2015).

At the level of the ovary, thyroid hormones play a role in growth and steroid hormone production. Triiodothyronine (T3) decreases E2 secretion from pre-recruitment follicles and the theca layer of pre-ovulatory follicles, while it also increases progesterone production from the granulosa layer of these follicles (Sechman, 2013). In comparative studies between feed restricted and full-fed broiler breeder hens, there were only differences in circulatory T3 levels before sexual maturation (Bruggeman et al., 1997; Sun et al., 2006), which may not explain dysregulation of egg production in feed restricted or full-fed adult birds. In the same studies, systemic levels of IGF-I and IGF-II were also found to be decreased in mature full-fed compared to restricted broiler breeders (Hocking et al., 1994; Bruggeman et al., 1997; Sun et al., 2006), but the source of IGF-I and the direct effect of these increased levels on the ovary was not studied. Full feeding broiler breeders 1 week before photostimulation increased hepatic and adipose lipogenic protein mRNA levels, whereas in feed restricted birds lipogenic protein mRNA increased only after photostimulation (Richards et al., 2003). The authors indicated that this may have been related to yolk lipid formation and as such had an effect on follicle growth. However, as photostimulation in this study occurred at the same time as an increase in feed allowance in the feed restricted birds, the effects of feed allocation and photostimulation itself could not be distinguished.

Adiponectin has been identified as a regulating factor in the effects of adiposity on reproduction (Mellouk et al., 2018). Under feed restriction, adiponectin expression is decreased in visceral fat (Maddineni et al., 2005; Bornelöv et al., 2018) and in juvenile broilers, plasma adiponectin levels are inversely related to abdominal fat pad mass (Hendricks et al., 2009). In laying hens, adiponectin and two adiponectin receptors (AdipoR1 and AdipoR2) mRNA transcripts were detected in granulosa and theca cells (Chabrolle et al., 2007). In addition, *in vitro* supplementation of human recombinant adiponectin increased IGF-I-induced progesterone secretion in granulosa cells of F2-F4 follicles and decreased LH- or FSH-induced progesterone production in granulosa cells of F3 and F4 follicles (Chabrolle et al., 2007). However, adiponectin was not differentially expressed comparing laying hens and broiler breeders at sexual maturity (Bornelöv et al., 2018). On the cellular level, viability of freshly isolated granulosa cells from hierarchical follicles was less in full-fed compared with feed restricted broiler breeders, where the proportion of cells undergoing apoptosis was 2 to 4 fold greater (Xie et al., 2012). The authors related the result to pathways involving palmitate-derived metabolites in an *in vitro* study, yet were unclear on how any systemic changes interacted on the cellular level *in vivo*. In laying hens, intra-

muscular injection of ghrelin increased progesterone release and also prevented feed-restriction induced decrease in ovarian testosterone and E2 (Sirotkin and Grossmann, 2015).

In addition, intra-muscular administration of obestatin increased ovarian progesterone production in both full-fed and feed restricted hens and ovarian E2 production in full-fed hens (Sirotkin and Grossmann, 2015). It was suggested that obestatin may be a mediator of the effects of feed restriction on ovarian hormones, as obestatin increased the effect of feed restriction on progesterone and testosterone. However, there is no cellular mechanistic explanation of a direct effect of obestatin or ghrelin on the ovary or the hypothalamus. In addition, the effects might differ between laying and broiler breeder hens.

Anti-Mullerian hormone (AMH) was also identified as a possible regulator of follicle recruitment (Johnson et al., 2009; Johnson, 2015). AMH is expressed in granulosa cells in the ovary, where the smallest follicles express the greatest amounts and decreasing amounts are expressed with increased follicle size (Johnson et al., 2009). The expression of AMH by granulosa cells is not under influence of E2 or progesterone, and AMH decreases FSH sensitivity in pre-recruitment follicles (Durlinger et al., 2001; Johnson et al., 2009). AMH mRNA expression is significantly greater in granulosa cells of pre-recruitment follicles of broiler breeder hens compared with laying hens (Johnson et al., 2009). In addition, full feeding broiler breeders increased follicle growth, which was associated with increased AMH mRNA expression (Johnson et al., 2009). Feeding level in broiler breeder hens is not associated with any difference in basal or surge amplitude of plasma LH or progesterone or the mean level of plasma E2 (Liu et al., 2004), therefore, it was suggested that AMH may be involved in follicle recruitment in the ovary and promote the appropriate and timely development of the response to FSH (Johnson et al., 2009). Feed restricted broiler breeder hens also have an increased expression of the FSH receptor in the F1 follicle compared to full-fed birds (McDerment et al., 2012). This suggests that feed restriction in broiler breeder hens results in a F1 follicle with greater responsiveness to stimulation by FSH. This is supported by findings from Lui et al. (Liu et al., 2014), where progesterone concentration was higher in the F1 of feed restricted compared to full-fed broiler breeders. They also investigated the linkage between leukocyte infiltration and function and reproductive performance and it was concluded that reproductive issues in full-fed broiler hens could be a result of obesity-associated metabolic dysfunction leading to deranged lipid metabolism termed “lipotoxicity” and an inflamed state of the ovary (Chen et al., 2006), mediated by ceramide, IL-1β, and other factors (Pan et al.,

2012). Table 2.5 provides an overview of the factors that may be part of the mechanisms explaining the differences in reproductive output between mature full-fed and restricted fed laying or broiler breeder hens.

2.3.3 Seasonal dynamics

2.3.3.1 Factors underlying sexual maturation

Under natural circumstances, environmental factors control seasonal breeding in birds. The most important ultimate factors are the availability of food for the hatchlings, availability of nesting sites, predation pressure, and climate factors (Ubuka and Bentley, 2011). As breeding takes place several weeks before chicks are hatched, the onset of maturation of the reproductive tract is started by proximate factors, such as photoperiod. The origin of the chicken can be traced back to the Red Jungle fowl, currently inhabiting an equatorial region in South East Asia. The need to respond to photoperiod might be of lesser importance in tropical circumstances around the equator, as food supply is relatively constant throughout the year and daylength variations are less pronounced. However, the Red Jungle fowl shows still a robust photoperiodic response (Ono et al., 2009). Domestication of chickens and the subsequent migration with humans around the globe could have imposed a shift in the proximate driving force of seasonality in chickens. Chickens are now inhabiting mid and high latitudes making photoperiod an important proximate factor to rely on for seasonality of reproduction. However, domestication and the supplying of food by the caretakers made availability of resources high throughout the year. The latter implies that there should be a reduced dependence on season for initiating reproduction. Indeed, several studies discussed a domestication related mutation in the thyroid stimulating hormone receptor gene (*TSHR*) and its modulation of photoperiodic response and reproduction (Rubin et al., 2010; Karlsson et al., 2016; Qanbari et al., 2019). Karlsonn et al. (2016) also reported that there was less responsiveness to photoperiod in the White Leghorn compared to the Red Jungle Fowl. This possibly resulted from stringent intensive selection for early onset of lay. There also appeared to be effects of domestication in the region of the TSHR gene for broiler lines (Rubin et al., 2010), but the functional meaning for broiler breeders is still poorly understood. Recently, a mutation was found in the *IGSF10* gene in broiler lines compared with layer genetic lines (Qanbari et al., 2019). The *IGSF10* gene is involved in reproduction, specifically in the migration of GnRH neurons during fetal development (Howard et al., 2016). Broiler breeders still strongly depend on photoperiodic cues for the onset of sexual maturation and reproduction, and display

photorefractoriness (Lewis, 2006), in contrast to laying hens (Morris et al., 1995; Lewis et al., 1997). High producing lines of laying hens can reach sexual maturity without photostimulation (Baxter and Bédécarrats, 2018), which implies other proximate factors might be involved in their initiation of sexual maturation, such as body weight, body composition, or nutrition (Bédécarrats et al., 2016). Quantitative breeding studies indicated a negative correlation between increased body weight and age at sexual maturity (Dunnington and Siegel, 1996; Jambui et al., 2017), meaning breeding for increased body weight delayed age of sexual maturity. Quantitative Trait Loci (QTL) for body weight and growth co-localise with the QTL for sexual maturity (Podisi et al., 2011), which indicates that breeding for body weight and growth rate can have a correlated effect on age at sexual maturity (Soller et al., 1984). Understanding the relevance of these QTL and potential strategies of genomic selection methods would enhance the ability to improve balanced selection for both growth and reproductive traits in broilers, including sexual maturation.

2.3.3.2 Photorefractoriness and dysregulation of sexual maturation

Photorefractoriness is the inability to respond to an otherwise stimulatory photoperiod and prevents animals from becoming sexually active when the existing environmental conditions are inopportune for successfully raising offspring (Lewis et al., 2003; Lewis, 2006). Juvenile photorefractoriness prevents broiler breeders from initiating reproduction in the same year in which they are hatched, even though they may be somatically mature (Lewis et al., 2004). The adult form of photorefractoriness causes gonadal regression (Lewis et al., 2010). Broiler breeders possess a relative form of juvenile photorefractoriness, which allows them to reach sexual maturity during their first year even though stimulatory photoperiodic cues are absent (Lewis et al., 2005). Under photostimulatory photoperiods during rearing, the dissipation of the photorefractory state is slow, and onset of sexual maturation and the age at first egg delayed compared with rearing under short photoperiods (Lewis et al., 2005; van der Klein et al., 2018b; Chapter 5). Chickens also possess a relative form of adult photorefractoriness, which means that after a prolonged exposure to a photostimulatory photoperiod, they do not ultimately stop egg production, but rather decrease the number of eggs produced (Sharp, 1993).

Although originally the effects of body weight, or feed allowance, and photoperiod on age at sexual maturity were considered to be independent (Lewis et al., 2004), recent evidence indicates there is an interaction of these factors in broiler breeders (van der Klein et al., 2018b; Chapter 5). In addition, broiler breeder hens do not respond uniformly to photostimulation before

the photorefractory state is dissipated, even at a mature body weight (van der Klein et al., 2018a; Chapter 4). One of the suggested causes for failure to reach sexual maturity after photostimulation in current lines of broiler breeders is a lack of adipose tissue (Zuidhof, 2018). This is in contrast to a previous conclusion that there is only a threshold for lean body mass for the onset of sexual maturation (Eitan et al., 2014). Genetic selection has increased growth rate and mature body weight, but has also increased lean tissue mass and decreased adiposity in broilers (Zuidhof et al., 2014). Relative fat pad weight (as % of body weight) was on average 0.41% at week 16 for the combined Ross 708, Ross 508 and Hubbard Hi-Y strains in 2007 (Robinson et al., 2007) and the relative fat pad weight was 0.10% for a line of Cobb grandparent females at the same age in 2018 (Zuidhof, 2018). In current lines of broiler breeders, an increase in body weight improved reproductive performance (van der Klein et al., 2018b; Chapter 5), which was likely due to increased adiposity. This is somewhat in contrast with conclusions described in the previous section, where it was suggested reproductive issues in mature broiler breeder hens are a result of a general state of 'lipotoxicity', i.e. a surplus of adipose tissue. Hence, there seems to be two separate issues: 1) the inability to acquire sexual maturity, related to a lack of adipose tissue resulting from increasingly restricted feeding practices and breeding goals aiming at minimizing adipose tissue deposition and 2) the inability to sustain a healthy reproduction after sexual maturity has been reached, related to a surplus of adipose tissue, especially when feed allocations are increased. However, the metabolic signals responsible for the communication between adipose tissue or hepatic tissue, and the reproductive system in birds are still mostly unknown. It has been suggested that leptin and hypothalamic NPY, modulated by the metabolic status, might be involved in controlling the onset of sexual maturation (Richards et al., 2010). However, recent cloning of the leptin gene and subsequent analysis of expression patterns suggested that leptin in chickens might have merely an autocrine or paracrine function, rather than the endocrine function as observed in mammals (Seroussi et al., 2016). There was a decreased GnRH-I expression in feed restricted broiler breeder hens (Ciccone et al., 2007), however, as previously concluded, the precise action of NPY on GnRH-I in both sexual maturation and during the reproductive phase will need to be clarified. The GnIH peptide could be another candidate for further exploration of the link between photorefractoriness and the effects of body weight on reproduction in broiler breeder hens. Voluntary reduction of feed intake in heat-stressed 14-day old layer line males resulted in an increase in GnIH mRNA expression in the diencephalon, but heat stress did not affect GnIH

mRNA expression in fasted birds (Bahry et al., 2018). This indicated that GnIH could be linked to appetite and nutrient intake. There might also be a role for steroidogenesis regulating genes *StAR*, *CYP11A1*, *HSD3B*, and *CYP19*, as these were 2 to 3-fold upregulated in the ovarian cortex of full-fed broiler breeders compared to their feed restricted counterparts at week 16 (Diaz and Anthony, 2013). Also, *BMP15* mRNA was decreased in full-fed compared to restricted fed pullets (Diaz and Anthony, 2013). This finding suggests that there is an increase in factors within the ovary related to early stage follicular growth and development in full-fed broiler breeders. Whether physiological differences between full-fed and feed restricted broiler breeder hens reduce the age at which sexual maturation is initiated or, reduces the time required to complete sexual maturation (from initiation to the lay of the first egg) still remains to be elucidated. Similarly, how metabolic status interacts with the response to photostimulation is still unclear.

2.3.4 Summary

Current meat-type chickens face challenges with reproductive abnormalities caused by weight and body composition control issues. The mechanisms behind diurnal and seasonal dynamics affecting egg production need to be further explored to understand and tackle these challenges. Diurnal dynamics of egg production in the chicken have been studied in detail, both mechanistically and endocrinologically, but the underlying proximate cause of ovulation and follicle recruitment has yet to be identified, which may be underlying the cause of reproductive issues in mature broiler breeders. In addition, there are still several missing links within our understanding of the neuroendocrinological factors playing a role in seasonal maturation processes, such as the causes of photorefractoriness and underlying metabolic signals, including the effect of feed restriction on hypothalamic maturation. There is also a significant amount of investigative work needed to clarify the effects of metabolic status on hypothalamic maturation and maturation of the ovary. The effects of metabolic status on maturation processes, signalling pathways, and feedback loops within the ovary or between the ovary and the hypothalamus might have been underestimated in previous studies and warrant further investigation.

2.4 Objectives and hypotheses

The above literature review concludes that mathematical modeling can be used as a tool to understand and study energy partitioning. Understanding energy partitioning is needed to make optimal use of resources for the overall sustainability of the poultry industry. In addition, controlling the energy balance in broiler breeders is needed for optimal egg production. Reproductive dysregulation in broiler breeders originates from a complex system of metabolic factors which may interact with other regulatory systems in the reproductive tract such as the sensitivity to photoperiod. Therefore, energy partitioning in meat-type chickens warrants further investigation with the use of modeling techniques. Of particular importance are individual, feed-source, and age-related variation in maintenance requirements, because explaining these sources of variation may allow for improved selection indicators and improved feed formulation and allocation practices. Furthermore, the interaction between the metabolic status and photosensitivity on the activation of the HPG-axis need further research, where there is need for a quantitative comparison of the dynamics of endocrinology compounds.

2.4.1 General objective

The objective of this thesis was to understand the effects of controlling energy intake and photoperiod on energy balance between maintenance, growth, and reproduction in meat-type chickens. The methods and topics studied in this thesis provided insights into best management practices for broiler (breeder) producers, poultry nutritionists, and primary breeding companies for optimizing their production efficiency, diet formulation, and breeding goals.

2.4.2 Specific objectives

- To validate a mathematical modelling methodology for energy partitioning to determine heat production and retained energy from metabolizable energy intake and for calculating net energy for gain values of feed in broilers (Chapter 3).
- To evaluate carcass composition, diet-specific heat increment of feeding, shank skin temperature, humoral immunological parameters, and activity level as potential causes for differences in total heat production in broilers (Chapter 3).
- To investigate the effects of body weight and age at photostimulation on broiler breeder reproductive performance (Chapter 4).
- To investigate the interaction between body weight and rearing photoperiod on broiler breeder reproductive performance (Chapter 5).

- To develop a mathematical model to describe estradiol-17β as a function of age, as a tool to compare estradiol-17β levels in a holistic and integrative manner and to provide scientific insight into estradiol-17β profiles and dynamics in broiler breeders (Chapter 6).
- To investigate the effect of body weight and rearing photoperiod on plasma estradiol-17β levels in broiler breeders (Chapter 6).
- To develop non-linear mixed models partitioning metabolizable energy intake to body weight, average daily gain, and egg mass during the complete broiler breeder production cycle, including individual, age-related, or both individual and age-related random terms to explain these sources of variation in heat production (Chapter 7).
- To investigate the interaction between body weight and rearing photoperiod on energy partitioning and energetic efficiency in broiler breeders (Chapter 7).

2.4.3 Hypotheses

- It was hypothesized that the mathematical model would estimate similar values for heat production and retained energy compared to the comparative slaughter technique including estimating a comparable net energy values for gain of the diets in broilers.
- It was hypothesized that broilers fed a low metabolizable energy diet would have a higher heat production at increased levels of feed intake compared to a high metabolizable energy treatment due to a higher heat increment of feeding.
- It was hypothesized that broiler breeders following a higher body weight profile would have an advanced onset of lay at the same age at photostimulation and therefore show an increased egg production, due to a lengthened laying period because of an earlier onset of lay.
- It was hypothesized that onset of lay would be delayed in lower body weight broiler breeders under extended photoperiods, and egg production would be reduced. Within photoschedule treatments, higher body weight birds would have an advanced onset of lay, thereby increasing total egg production.
- It was hypothesized that the mathematical model would show biological insight into the dynamics of estradiol-17β concentration during sexual maturation in response to body weight and rearing photoperiod in broiler breeders.
- It was hypothesized that broiler breeders following a higher body weight would have a shorter duration between photostimulation and the estradiol-17β-inflection point, but that

time at highest rate of estradiol-17β increase would be consistent between treatments relative to the onset of lay.

- It was hypothesized that the energy partitioning model for broiler breeders including random terms for both individual and age-related heat production would fit the data best.
- It was hypothesized that feed conversion ratio for gain, feed conversion ratio for egg mass produced, residual feed intake, and residual heat production would be decreased in broiler breeders on treatments with reduced photoperiod and reduced body weight.

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2.6 Tables

Table 2.1 Overview of standardized to 1.5 kg and reported ME requirements for maintenance (ME_m) of mature and pullet broiler breeders (BB) and egg-type (ET)[1] chickens.

Standardized ME_m requirement (kcal/$kg^{0.67}$)	Reported value	Unit of reported value	Bird and age	Housing system	Method	Reference
116.4	113.0	kcal/$kg^{0.75}$	BB mature	group	CST	Rabello et al. (2006)
160.7	1.6	kcal/$g^{0.67}$	BB mature	group	Mathematical model	Pinchasov and Galili (1990)
90.7	87.8	kcal/$kg^{0.75}$	BB mature	individual	Indirect calorimetry	Spratt et al. (1990)
101.5	98.3	kcal/$kg^{0.75}$	BB mature	individual	Mathematical model	Reyes et al. (2011)
104.1	100.8	kcal/$kg^{0.75}$	BB mature	individual	Mathematical model	Reyes et al. (2012)
107.8	104.4	kcal/$kg^{0.75}$	BB mature	individual	Mathematical model	Romero et al. (2009b)
111.4	104.1	kcal/$kg^{0.84}$	BB mature	individual	Mathematical model	Pishnamazi et al. (2015)
110.0	106.5	kcal/$kg^{0.75}$	BB mature	individual	Mathematical model	Romero et al. (2011)
132.7	139.9	kcal/$kg^{0.54}$	BB mature	individual	Mathematical model	Romero et al. (2009a)
133.1	140.3	kcal/$kg^{0.54}$	BB mature	individual	Mathematical model	Romero et al. (2009a)
139.1	134.7	kcal/$kg^{0.75}$	BB pullet	group	CST, kg RE/(MEI-ME_m)	Sakomura et al. (2003)
150.6	145.8	kcal/$kg^{0.75}$	BB pullet	group	CST, kg slope of RE and MEI	Sakomura et al. (2003)
111.5	111.0	kcal/$kg^{0.68}$	BB pullet	group	Mathematical model	Hadinia et al. (2018)
129.5	129.0	kcal/$kg^{0.68}$	BB pullet	group	Mathematical model	Hadinia et al. (2018)
122.0	122.0	kcal/$kg^{0.67}$	BB pullet	group	Mathematical model	Zuidhof et al. (2017)
99.3	103.0	kcal/kg	BB pullet	unknown	Mathematical model	Darmani Kuhi et al. (2011)
88.0	85.2	kcal/$kg^{0.75}$	ET mature	individual	Indirect calorimetry	Johnson and Farrell (1983)
84.9	82.2	kcal/$kg^{0.75}$	ET mature	individual	Indirect calorimetry	Johnson and Farrell (1983)
107.4	104.0	kcal/$kg^{0.75}$	ET mature	individual	Mathematical model	Valencia et al. (1980)
84.8	88.0	kcal/kg	ET mature	unknown	Mathematical model	Darmani Kuhi et al. (2012)
75.4	73.0	kcal/$kg^{0.75}$	ET pullet	individual	Indirect calorimetry	Johnson and Farrell (1983)
76.5	74.0	kcal/$kg^{0.75}$	ET pullet	individual	Indirect calorimetry	Johnson and Farrell (1983)
165.8	1.62	kcal/g	ET pullet	unknown	Mathematical model	Hurwitz et al. (1978)

[1]Average standardized ME_m requirements were 116.8 ± 20.2 kcal/$kg^{0.67}$ for mature BB, 125.3 ± 18.6 kcal/$kg^{0.67}$ for BB pullets, 91.3 ± 10.9 kcal/$kg^{0.67}$ for mature LH, and 105.9 ± 51.9 kcal/$kg^{0.67}$ for pullet LH.

Table 2.2 Overview of ME requirements for gain of mature and pullet broiler breeders (BB)[1], egg-type pullets, and broilers

ME requirement for gain (kcal/g)	Housing system	Bird	Age (wk)	Reference
3.36	individual cage	BB mature	20 to 60	Romero et al. (2009b)
1.89	individual cage	BB mature	16 to 60	Romero et al. (2009a)[2]
5.80	individual cage	BB mature	34 to 44	Reyes et al. (2012)
2.13	individual cage	BB mature	25 to 41	Pishnamazi et al. (2015)
3.39	individual cage	BB mature	20 to 56	Romero et al. (2011)
1.52	group housed	BB pullet	10 to 23	Hadinia et al. (2018)
0.71	group housed	BB pullet	0 to 16	Pishnamazi et al. (2008)
2.83	individual cage	BB pullet	3 to 8	Sakomura et al. (2003)
2.50	individual cage	BB pullet	9 to 14	Sakomura et al. (2003)
3.24	individual cage	BB pullet	15 to 20	Sakomura et al. (2003)
0.71	group housed	BB pullet	3 to 20	Pinchasov and Galili (1990)
2.05	unknown	Broiler	1 to 14	Hurwitz et al. (1978)
1.87	individual cages	Broiler	5 to 25	Hurwitz et al. (1980)
2.19	unknown	ET pullet	1 to 14	Hurwitz et al. (1978)

[1]Average ME requirements for gain were 3.41 ± 1.45 kcal/g for mature BB, 1.92 ± 1.09 kcal/g for BB pullets.
[2]Based on a 1.5 kg bird growing at 10 g per day.

Table 2.3 Overview of ME requirements for egg mass production (EM) of broiler breeders (BB) and egg-type hens (ET)

ME requirement for egg production (kcal/g egg)	Bird	Reference
3.10	BB	Combs (1968)
1.79	BB	Pishnamazi et al. (2015)
2.40	BB	Rabello (2001)
2.30	BB	Reyes et al. (2012)
2.02 to 2.37	BB	Romero et al. (2009a)[1]
1.92	BB	Sakomura (1989)
2.08	BB	Romero et al. (2011)
2.01	ET	Marsden and Morris (1987)
2.07	ET	NRC (1994)
2.40	ET	Sakomura et al. (2005)

[1] Based on average EM and average BW calculated with 3 different models.

Table 2.4 Overview of the steroids produced by the different cell layers in follicles in the ovary of the mature chicken in their respective maturation stage and the pathways involved. A plus (+) indicates that the cell layer produces or is responsive to the component/pathway, a minus (-) indicates that components are not find or affecting the cell layer. Derived from Robinson and Etches (1986), Johnson (2015), and Ghanem and Johnson (2019a).

		Intermediates[1]		**Output[2]**			**Gonadotropic responsiveness[3]**		**Pathways**		
Cell layer	**Stage**	**P5**	**DHEA**	**P4**	**A**	**E2**	**FSH[5]**	**LH**	**P450arom[4]**	**Δ5**	**Δ4**
Granulosa	Primordial	-	-	-	-	-	-	-	-	-	-
	Pre-recruitment	-	-	-	-	-	+	-	-	-	-
	Pre-ovulatory	-	-	++	-	-	++	++	-	+	++
	F1	-	-	+++	-	-	-	+++	-	+	+++
Theca externa	Primordial	-	-	-	-	+++	+	+++	+++	+	+
	Pre-recruitment	-	-	-	-	++	++	++	++	+	+
	Pre-ovulatory	-	-	-	-	+	+	+	+	+	+
	F1	-	-	-	-	-	-	-	-	+	+
Theca interna	Primordial	+++	+++	-	+++	-	+	+++	-	+++	+
	Pre-recruitment	++	++	-	++	-	++	++	-	++	+
	Pre-ovulatory	+	+	-	+	-	+	+	-	+	+
	F1	+	+	+	+	-	-	-	-	+	+

[1] P5 = pregnenolone, DHEA = dihydroepiandrosterone
[2] P4 = progesterone, A = androstenedione, E2 = estradiol-17β
[3] FSH = follicle stimulating hormone, LH = luteinizing hormone
[4] P450arom = P450 aromatase
[5] FSH receptor desensitized in primordial granulosa cells, but progressively activated with increasing follicle size.

Table 2.5 Overview of potential (metabolic) factors and mediators underlying the differences in reproductive output between full or restricted fed laying hens and broiler breeders.

Factor/ Mediator[1]	Site	Location	Biological effect[2]	Reference
T3	Ovary	Theca layer pre-ovulatory follicle	Decreased E2 secretion in Lh *in vitro*; increased circulatory T3 levels in juvenile full-fed vs restricted Bb	Bruggeman et al., 1997; Sun et al., 2006; Sechman, 2013
T3	Ovary	Granulosa pre-recruitment and pre-ovulatory follicle	Increased concentration of progesterone in Lh *in vitro*; increased circulatory levels in juvenile full-fed vs restricted Bb	Bruggeman et al., 1997; Sun et al., 2006; Sechman, 2013
Ghrelin	Ovary	-	Increased progesterone release and prevented feed-restriction induced decrease in testosterone and E2 in Lh	Sirotkin and Grossmann, 2015
NPY	Hypo-thalamus	Median Eminence	Increased release of GnRH-I during preovulatory release of Lh; genetic association with egg production and age at sexual maturity.	Dunn et al., 2004, 2009; Wu et al., 2007
AMH	Ovary	Pre-recruitment follicles	Decreases FSH sensitivity; mRNA expression greater in granulosa cells of Bb hens compared to Lh; increased AMH mRNA expression in follicles of full-fed vs restricted Bb	Johnson et al., 2009; McDerment et al., 2012
IGF-I	Ovary	-	Enhance proliferation of theca externa cells in Lh; circulatory levels decreased during rearing and increased at sexual maturity in restricted vs full-fed Bb	Bruggeman et al., 1997; Sun et al., 2006; Jia et al., 2013
FSH receptor	Ovary	F1	Increased expression in feed restricted vs full-fed Bb	McDerment et al., 2012
Progesterone	Ovary	F1	Higher concentration in feed restricted vs full-fed Bb	Liu et al., 2014
Leukocytes, inflammatory cytokines	Ovary	F1	Increased infiltration with immune cells, increased IL-1β content, and reduced MMP activity in full-fed vs restricted Bb	Pan et al., 2012; Liu et al., 2014
BMP15	Ovary	Ovarian cortex	Decreased at week 16 in full-fed vs restricted Bb	Diaz and Anthony, 2013
Steroidogenesis regulating genes[3]	Ovary	Ovarian cortex	Increased at week 16 in full-fed vs restricted Bb	Diaz and Anthony, 2013
Adiponectin	Ovary	Granulosa cells	Increased IGF-I-induced progesterone secretion in F2-F4 and decreased LH- or FSH-induced progesterone production of F3 and F4	Maddineni et al., 2005; Chabrolle et al., 2007; Hendricks et al., 2009; Mellouk et al., 2018
Lipogenic genes[4]	Liver	-	In general, an increase pre-photostimulation and decrease at first egg in full-fed vs restricted Bb	Richards et al., 2003

[1]T3 = Triiodothyronine; NPY = Neuropeptide Y; AMH = Anti-Mullerian hormone; IGF = Insuline-like growth factor I; FSH = Follicle stimulating hormone, BMP = Bone morphogenic protein.
[2] Lh = Laying hen, Bb = Broiler breeder, MMP = matrix metalloproteinase (catalyzes tissue matrix degradation leading to follicle rupture)
[3]StAR (steroidogenic acute regulatory protein), CYP11A1 (cholesterol side-chain cleavage enzyme), CYP19 (P450 aromatase), HSD3B (3β-Hydroxysteroid dehydrogenase/Δ5-4 isomerase)
[4]Sterol regulatory element binding protein-1, cytosolic malic enzyme, acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA (Δ9) desaturase-1.

2.7 Figures

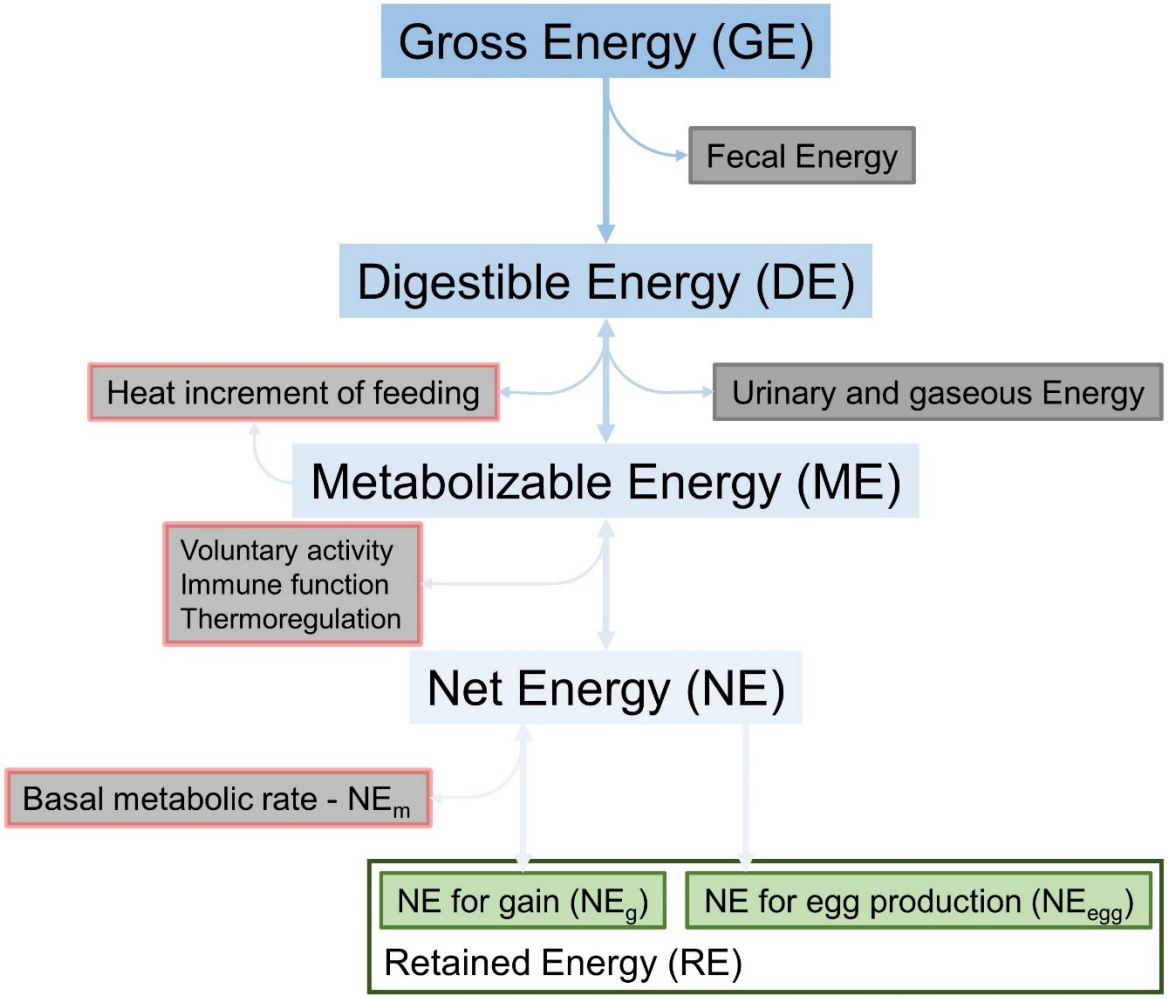

Figure 2.1 Overview of the energy partitioning framework based on (NRC, 1981a; Zuidhof, 2019). Blue boxes represent energy pools, grey outlined grey boxes represent unavailable energy, red outlined grey boxes represent energy eventually lost as heat, and the green boxes represent productive energy. Bidirectional arrows indicate transformation of energy can take place in both directions.

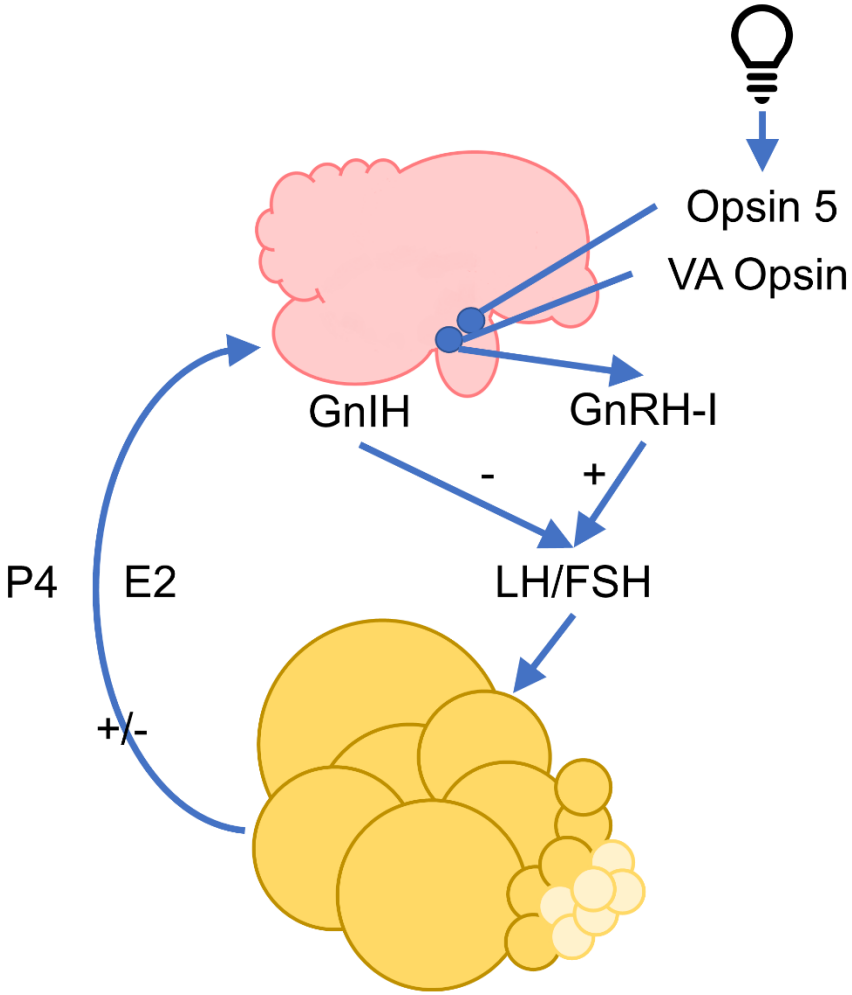

Figure 2.2 Schematic overview of the hypothalamus-pituitary-gonadal axis. GnIH = Gonadotropin inhibitory hormone, GnRH-I= Gonadotropin releasing hormone I, LH = luteinizing hormone; FSH = follicle stimulating hormone, P = Progesterone, E2 = Estradiol-17β. Blue circles indicate the paraventricular organ, pink area indicates the brain, and the yellow circles indicate the ovary. GnIH and GnRH are released from the hypothalamus and inhibit/stimulate the release of LH and FSH from the pituitary into the circulatory system Based on results from Robinson and Etches (1986), Dunn and Sharp (1990), Kirby et al. (2005), and Bédécarrats et al. (2016).

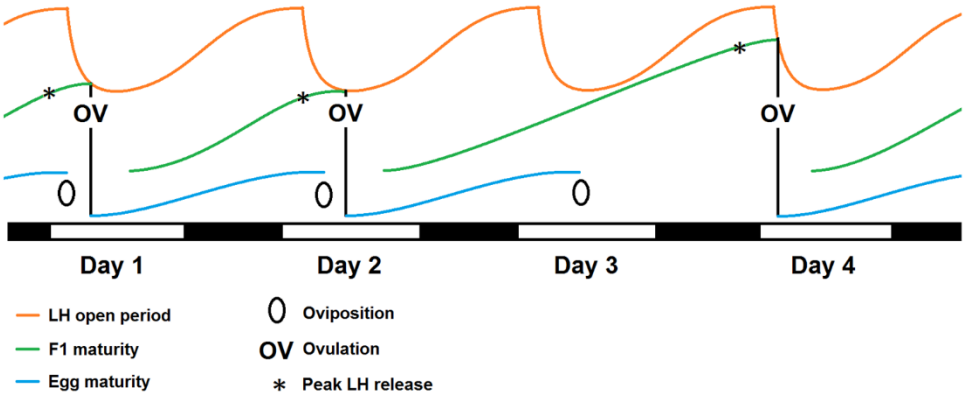

Figure 2.3 Diurnal rhythmicity in the photoperiod entrained LH open period, F1 degree of maturity, egg degree of maturity, peak LH release, ovulation and oviposition. White areas on the x-axis indicate photoperiod, dark areas on the x-axis indicate scotoperiod. Adapted from Fraps, (1965) and Etches (1990).

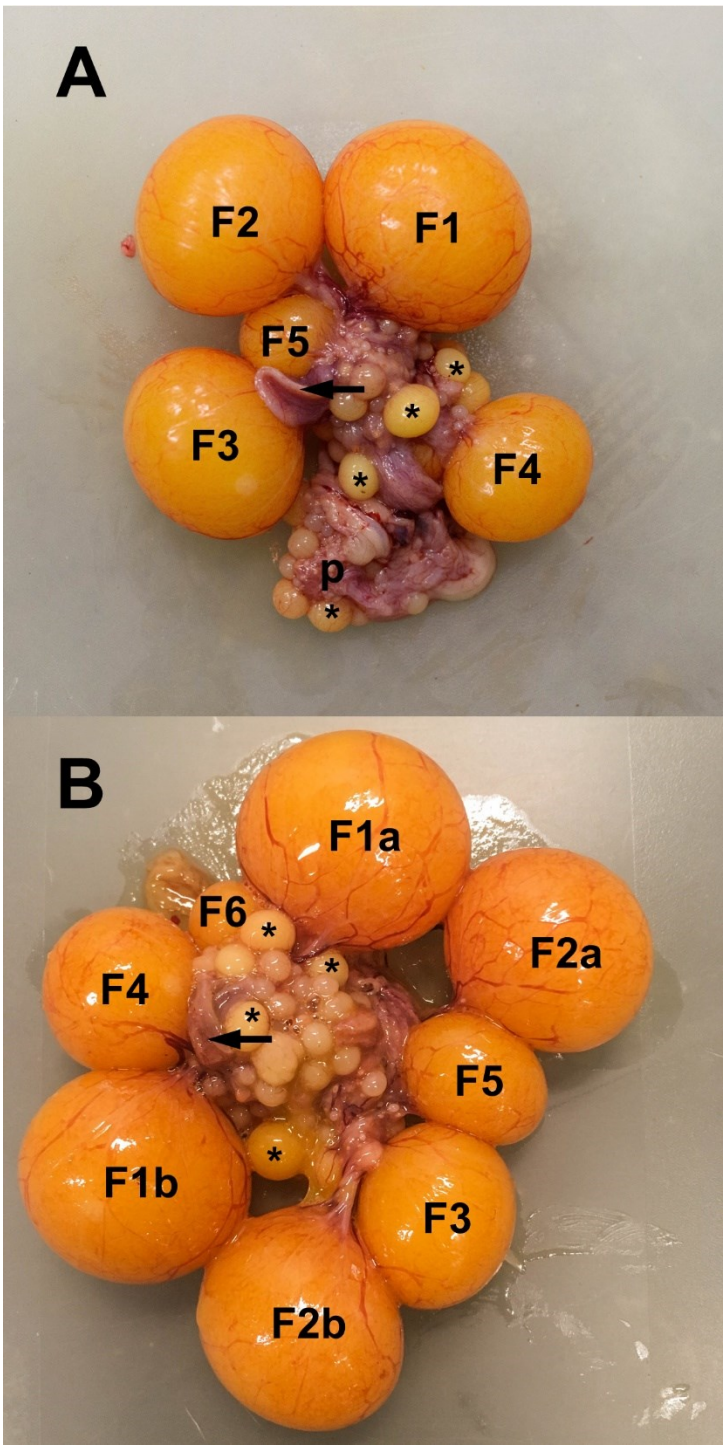

Figure 2.4 Ovary of a restricted fed (A) and full-fed (B) broiler breeder. Arrows indicate post-ovulatory follicles, asterisk (*) indicate examples of pre-recruitment follicles, p indicates primordial follicles, and pre-ovulatory follicles are indicated F1 to F6, with a and b indicating follicles identical in size (double hierarchy).

CHAPTER 3.

Comparison of mathematical and comparative slaughter methodologies for determination of heat production and energy retention in broilers[2]

3.1 Abstract

Understanding factors affecting ME availability for productive processes is an important step in optimal feed formulation. This study compared a modelling methodology with the comparative slaughter technique (CST) to estimate energy partitioning to heat production (HP) and energy retention (RE) and to investigate differences in heat dissipation. At hatch, 50 broilers were randomly allocated in one of 4 pens equipped with a precision feeding station. From d 14 to d 45 they were either fed a Low ME (3,111 kcal/kg ME) or a High ME (3,383 kcal/kg ME). At d 19, birds were assigned to pair-feeding in groups of 6 with lead birds eating ad libitum (100%) and follow birds eating at either 50%, 60%, 70%, 80%, or 90% of the paired lead's cumulative feed intake. HP and RE were estimated by CST and with a non-linear mixed model explaining daily ME intake (MEI) as a function of metabolic BW and daily gain (ADG). The energy partitioning model predicted MEI = (145.10 + u) $BW^{0.83}$ + 1.09 × $BW^{-0.18}$× $ADG^{1.19}$ + ε. The model underestimated HP by 13.4% and overestimated RE by 22.8% compared to the CST. The model was not able to distinguish between net energy for gain (NEg) values of the diets (1,448 ± 18.5 kcal/kg vs 1,493 ± 18.0 kcal/kg for the Low ME and High ME diet, respectively), whereas the CST found a 148 kcal/kg difference between the Low ME and High ME diets (1,101 ± 22.5 kcal/kg vs 1,249 ± 22.0 kcal/kg, respectively). The estimates of the NEg values of the two diets decreased with increasing feed restriction. The heat increment of feeding did not differ between birds fed the Low or High ME diet (26% of MEI). Additional measurements on heat dissipation, physical activity, and immune status indicated that the energetic content of the diet and feed restriction affect some parameters (shank temperature, feeding station visits), but not others (leukocyte counts, H:L ratio, and immune cell function).

[2] A version of this chapter has been submitted for publication in Poultry Science co-authored by J.A. More-Bayona, D.R. Barreda, L.F. Romero, M.J. Zuidhof

3.2 Introduction

Efficient and sustainable poultry production requires accurate estimation of productive (retained) energetic values of feed ingredients and complete diets. Currently, metabolizable energy corrected for zero nitrogen retention (MEn) is the most commonly used energy value for ingredients and diets for the broiler industry. There is an ongoing debate between researchers on whether the industry would benefit from a net energy (NE) system over the ME system (Wu et al., 2018; Zuidhof, 2019), because of misunderstanding of definitions and disagreement on whether a NE system would enhance efficiency and profitability of diet formulation. As defined by Fraps and Carlyle (1939) and later by NRC (1981), the NE for gain value for a feed (NEg), also called productive energy, is defined as the amount of energy stored by the chicken for a given amount of feed fed above that necessary for maintenance requirements. ME used for maintenance (MEm) is equivalent to total heat production (HP; NRC, 1981; Fraps and Carlyle, 1939; Latshaw and Moritz, 2009), therefore, NEg value for feed is equal to ME minus total HP from all sources divided by the weight of the feed consumed (Zuidhof, 2019). The purpose of the NEg value for feed is to characterize the quantity of the energy in the feed that is retained in the body and not released as heat. NEg is a property of the feed and expressed for example as kcal/kg.

Minimizing HP at the same level of ME intake will maximize the availability of energy for energy retention (RE; Zuidhof, 2019). Traditionally, it has been assumed that the energy requirement per unit of growth (g) is constant (Spratt et al., 1990; Rabello et al., 2006). Yet, depending on the composition and efficiency of energy retention (e.g. fat vs lean tissue), energy partitioned to gain changes (Kielauowski, 1965). In addition, total HP can depend on the ingredients and composition of the diet. For example, the efficiency of the use of ME for gain was 45.4 kcal per 100 g gain greater in birds fed diets containing sunflower oil than in those fed isoproteic tallow-containing diets, which could be the result of a reduction in HP (Sanz et al., 2000). Also, dietary NEg increased by 12.5% with supplementation of plant extracts carvacrol, cinnamaldehyde, and capsicum which was hypothesized to result from a change in intestinal microbiome (Bravo et al., 2014). In addition, RE increased from 53.4 kcal/d in a control diet deficient in ME to 70.3 kcal/d in the same diet supplemented with phytase (Olukosi et al., 2008).

To evaluate the effects of animal, dietary, or environmental effects on HP, the partitioning of ME intake to HP and RE needs to be estimated. Total HP can be calculated indirectly by measuring RE through the comparative slaughter technique (CST; Fraps, 1946). HP can be also

estimated through respiration calorimetry (Birkett and de Lange, 2001; Wu et al., 2018). Romero et al. (2009) proposed a mathematical method based on work by Byerly et al. (1980) and Schulman et al (1994), not assuming linearity and adjusting the energy requirement per unit of gain at different rates of gain. Mathematical modelling methods would be less invasive and less expensive as they do not require euthanizing animals (CST) or keeping them in respiratory units (respiration calorimetry). It would also be possible to relate estimated HP to ME intake per unit of metabolic BW and calculate a diet-specific heat increment of feeding (HIF), by comparing the slopes of the linear regression of individual HP on ME intake of different diets (Romero et al., 2011). Increased feed intake increases HIF (Liu et al., 2017). HIF is often expressed as a percentage of ME intake or in kcal, and part of total HP (NRC, 1981). As level of feed intake can vary between individuals, quantifying ingredient- and nutrient-specific change in HIF can be an important measure to explain a portion of the HP that causes variation in ME availability for RE. Higher feed intake or higher ME intake in broilers fed diets with increased ME:CP ratio have led sometimes to increased total HP (Buyse et al., 1992), whereas others found reduced total HP (MacLeod, 1997). HIF has also been suggested to regulate voluntary feed intake in broilers (Swennen et al., 2004), however, this could not yet be confirmed (Swennen et al., 2006, 2007). Yet, the literature has not studied diet specific HIF at different levels of ME intake or diet composition. It was suggested that HIF would be higher for diets with a low ME:CP ratio at higher levels of intake. Overconsumption of CP over ME could result in deamination of excess amino acids releasing heat and an energy source for the bird (Musharaf and Latshaw, 1999; Gous and Morris, 2005).

Hence, the objective of this study was two-fold, 1) to evaluate the accuracy of a novel mathematical modelling methodology for energy partitioning to determine HP, RE, and NEg from ME intake compared to the CST and 2) to estimate diet-specific HIF by comparing the slope of the linear regression of HP on ME intake of two energetically different diets. It was hypothesized that the mathematical model would estimate similar values for HP and RE compared to the CST, including estimating a comparable NEg value of the diets. In addition, it was hypothesized that birds fed the Low ME diet would have a higher HIF at increased levels of feed intake compared to the High ME treatment. Physiological adaptations affecting ME partitioning were investigated, which included evaluation of shank skin temperature and humoral immunological parameters. Body composition and feeding station visit frequency were evaluated to study the underlying potential causes of differences in total HP.

3.3 Materials and methods

3.3.1 Experimental design

The animal protocol for this study was approved by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care Guidelines and Policies (CCAC, 2009). The experiment was conducted as randomized block design of a 2 × 6 factorial arrangement of treatments with 50 broilers in 4 pens (blocks) fed an isonitrogenous Low ME (3,111 kcal/kg ME) or a High ME (3,383 kcal/kg ME) grower diet from d 14 and were provided ad libitum feeding or received 50, 60, 70, 80, 90% of ad libitum from d 19. The main experimental design was n = 25 per diet treatment with groups of birds fed at different levels. Pens were randomly assigned to the Low ME or High ME grower diet and birds within pens were randomly assigned restriction treatments. Individual bird was used as experimental unit.

3.3.2 Animals and housing

Day old Ross x Ross 308 feather sexed male broilers purchased from Lilydale Hatchery (Edmonton, Alberta, n = 50), were randomly allocated in one of four wood shavings covered floor pens, all equipped with a precision feeding (PF) station allowing individual feed distribution in a group housed setting, for detailed information see Zuidhof et al. (2016, 2017). At placement, birds were neck tagged for individual identification and trained to use the stations from 0 to 10 d of age. During this time, feeder space was limited. From d 0 to d 13 a starter diet was provided ad libitum. From d 14 to d 45 grower diets were fed at different levels using a precision feeding station. At d 10, birds received a radio frequency identification (RFID) tag and were transitioned to individual feeding, which was fully implemented at d 14. To create a robust model, a wide range of energy intakes were implemented. At d 19, two birds per pen were assigned to ad libitum treatment and used as lead birds. Ten other birds per pen were coupled randomly to one of the two lead birds per pen, and received either 50, 60, 70, 80, or 90% of its lead's cumulative feed intake, creating graded levels of energy intake.

3.3.3 Experimental diets

Diets were formulated on a least cost basis and comparable to commercially available wheat-soybean meal based diets in the Canadian Prairie Provinces. The ingredient composition and calculated and analyzed ME and nutrient content of the starter and grower diets is shown in

Table 3.1. Celite was used as an index for digestibility determination to determine ileal energy digestibility.

3.3.4 Data collection

From d 0 to d 13, birds were weighed manually on a daily basis to ensure growth and verify the use of the PF system. Birds that were not gaining weight or were gaining weight slowly were trained individually. After individual feeding had been fully implemented at d 14, the PF system recorded individual BW and feed intake on a per visit basis. Feed intake and visit frequency was checked on a daily basis to ensure all birds were accessing the PF system. Shank temperature measurements were taken from all birds on d 22, 28, 35, and 42 with a handheld infrared camera. The highest temperature detected by the camera was recorded, focused on the posterior side of the shank area. The camera recorded the exact time the temperature measurement was taken and this was aligned with feed intake data from the PF system. At d 45, 3 mL blood samples were collected in EDTA coated vacutainer tubes from the brachial vein of each bird and shortly after, all birds were killed by cervical dislocation. All birds that died or were culled during the experiment were recorded (n = 1). Abdominal fat pad (including fat adhering to the proventriculus and gizzard), filled gastro-intestinal tract (GIT), breast muscle (combined pectoralis major and pectoralis minor), heart, legs without skin (combined thigh and drum), and liver weight were recorded during the dissection. Intestinal content was collected from the distal part of the ileum and stored at -20°C prior to analysis. After removal of all intestinal content, the empty GIT was weighed. The GIT consisted of the complete digestive tract including pancreas, from 2 cm anterior to the crop up to but not including the bursa, with fat adhering to the proventriculus and gizzard removed. The sex of each bird was confirmed by visual inspection of the gonads at the time of dissection. All the dissected parts including empty carcass were collected in plastic bags and stored by -20°C prior to further sample processing.

3.3.5 Carcass and digesta composition analysis

Following pressure cooking and grinding of complete carcass, representative subsamples were taken and stored at -20°C prior to proximate analysis. Samples were dried at 60°C to determine carcass moisture. Dried samples were reground in a coffee grinder before energy content measurement and proximate analysis. Duplicate 1 g pellets of dried carcass sample were analyzed for energetic content in a bomb calorimeter (IKA Calorimeter System with C5000 control). Carcass samples were analyzed in duplicate for determination of total carcass dry matter, crude

protein, lipid, and ash using standard chemical analysis procedures (Horwitz, 1980). Ileal digesta samples were pooled per restriction treatment within diet treatment. Dried ileal digesta samples and feed samples were analyzed following the same protocol. Additionally, acid insoluble ash was analyzed in ileal digesta and feed samples. Samples were burned at 500°C overnight and then hydrolyzed with 4 M HCl at 110°C for 2 h. After centrifuging at 3000 rpm for 8 minutes at 20°C supernatant was discarded and ash was burned overnight at 500°C. Acid insoluble ash was calculated as the weight of the ash divided by the dry matter weight of the initial sample times 100%. The ME value of the diets was calculated by the following equation (Scott and Boldaji, 1997):

$$\mathrm{ME} = \mathrm{GE}_{\mathrm{feed}} - \mathrm{GE}_{\mathrm{digesta}} \times \frac{\mathrm{AIA}_{\mathrm{feed}}}{\mathrm{AIA}_{\mathrm{digesta}}}$$

where GE is the gross energy (kcal/kg) of the sample and AIA is the concentration of acid insoluble ash in the sample, all expressed on a dry matter basis.

3.3.6 Leukocytes

Peripheral blood leukocyte composition analysis was only performed on samples from the most extreme feed intake treatments; the 50% feed restricted and *ad libitum* fed birds. Directly after collection, blood smears were stained using the Hema3 staining set (Fisher Scientific) according to the manufacturer's specifications. Slides were air dried prior to observation using bright field microscopy. Photomicrographs were taken using a Leica DM1000 microscope and images were acquired using QCapture software. Two hundred and fifty cells were counted to estimate the heterophil/lymphocyte ratio.

3.3.7 Energy partitioning methods

Two methods were used to determine HP and RE in this study, the CST and a mathematical model explaining energy intake as a function of BW and gain. For the CST carcass gross energy content at d 14 was estimated from individual live weight using the regression equations from Wolynetz and Sibbald (1985) based on 10 d old broilers, where total carcass energy (kcal) = -181.2 kcal + 1,995.9 kcal/kg × BW (kg). For each individual RE was calculated by subtracting the estimated carcass gross energy content at d 14 from the measured carcass gross energy content at d 45. Individual total HP was calculated as follows:

$$\mathrm{HP\ (kcal)} = [\mathrm{FI\ (g)} \times \mathrm{ME}_{\mathrm{diet}}\ (\mathrm{kcal/g})] - \mathrm{RE\ (kcal)}$$

where FI is feed intake (g) over the experimental period (d 14 to d 45) and ME_{diet} is the analyzed ME content of the diet (kcal/g). The mathematical model used to predict energy partitioning to HP and RE was based on previous work of Romero et al. (2009) and used by others (Pishnamazi et al., 2008; Hadinia et al, 2018). The following model was defined in the NLMIXED procedure in SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012): $MEI_d = (a + u) \times BW^b + c \times BW^d \times ADG^e + \varepsilon$, $u \sim N(0, V_u)$, $MEI_d \sim N(\mu,V)$, where MEI_d = daily ME intake (kcal/d), BW = body weight (kg), ADG = average daily gain (g/d) calculated over a 4 d period, ε = residual error. The random term *u* was associated with each bird, variance parameters *V* and *Vu* were estimated in the regressions. The estimated equation was: $MEI_d = (145.10 + u)\ BW^{0.83} + 1.09 \times BW^{-0.18} \times ADG^{1.19}$ ($P < 0.001$ for all parameters; Table 3.2). The first part of the equation, $(145.10 + u) \times BW^{0.83}$, represented the partitioning of the daily ME intake towards maintenance, i.e. HP. The second part of the equation, $1.09 \times BW^{-0.18} \times ADG^{1.19}$, reflected the partitioning of daily ME intake towards gain, i.e. RE. Estimated HP and RE per 4-day period were summed to reflect total HP and total RE over the experimental period (d 14 to d 45). For both the CST as the model method, NE_g of the diets (kcal/kg) was calculated by dividing RE by the cumulative feed intake over the experimental period.

3.3.8 Statistical analysis

Animals that had to be culled before the end of the experiment because of a neurological abnormality (crooked neck, n = 1), and sexing errors (females, n = 2) were removed from the dataset for all analyses. All ANOVA were conducted using the MIXED procedure of SAS with the Kenward-Roger method specified in the DDFM option (Version 9.4. SAS Institute Inc., Cary, NC, 2012). Tukey's range test was used to compare treatment means and differences were considered significant at $P \leq 0.05$. The model used for BW at d 45, cumulative feed intake, cumulative energy intake, cumulative gain, and cumulative feed conversion ratio included diet treatment, feed restriction treatment, and their interaction as fixed effects. The model used for dissection and carcass composition data included diet treatment, feed restriction treatment, and their interaction as fixed effects and BW as a covariate to account for BW differences. The model used to determine difference in HP and RE between the diets, the difference between the mathematical model and CST method in HP and RE included the diet treatment, and the difference between NE_g included the diet treatment and feed restriction treatment and their interaction as fixed effects. The difference in HIF between the diets was tested by evaluating the slope of the

linear regression of individual daily HP per metabolic BW on average daily ME intake per metabolic BW for the modeling method. The first iteration used a model including diet treatment as fixed effect, ME intake as a covariate, and their interaction. Because the interaction was not significant for either methods, it was omitted from the model, and the results show the equation with diet treatment as a fixed effect, and ME intake per metabolic BW as a covariate. The model used to test the effect of diet treatments on shank temperature included age, diet treatment, and their interaction as fixed effects, and a covariate for ME intake during the 6, 12, 24, or 48 hours prior to the temperature measurement. The model used to test differences between the number of station visits per 24 h, the number of meals per 24 h, the meal to visit ratio, and meal size, included age, diet treatment, and feed restriction treatment as fixed effects, and all their interactions. The model used to test differences in percentages of leukocytes included diet treatment as a fixed effect and ME intake from d 14 to d 45 as a covariate.

3.4 Results and discussion

3.4.1 Diet analysis and bird performance

The analyzed ME content of the grower diets was higher than the originally calculated composition (Table 1, 3,111 vs 2,900 kcal/kg for the Low ME diet and 3,383 vs 3,150 kcal/kg for the High ME diet, respectively). The diets were formulated on MEn basis. The differences between analyzed and formulated energy levels could have resulted from variation in feed ingredients ME or be part of the nitrogen correction. However, as it was intended to create a difference in ME and the actual ME difference between the diets was 272 kcal/kg, it was not expected that this would alter the inference. BW at d 45 did not differ between birds fed the High ME diet and the Low ME diet (Table 3.3). This is consistent with results from Leeson et al. (1996), who found that BW at d 49 did not differ between *ad libitum* fed broilers fed diets ranging in ME between 2,700 kcal/kg and 3,300 kcal/kg. As anticipated, restricting feed intake reduced BW and gain to d 45 (Table 3, $P < 0.001$). Cumulative feed intake from d 14 to d 45 was lower in birds fed the High ME diet compared to birds fed the Low ME diet (2,988 g vs 3,099 g, $P = 0.047$). However, cumulative ME intake was higher in the High ME treatment compared to the Low ME treatment (10,108 kcal vs 9,641 kcal, $P = 0.012$). Earlier studies concluded that broilers were able to control their feed intake in *ad libitum* situations based on desire to normalize energy intake (Leeson et al., 1996), hence, with an increment of dietary ME, feed intake was reduced. Other studies concluded that broiler fed a diet with a higher ME:CP ratio overconsumed ME to meet CP requirements (Swennen et al.,

2004). As the diets were isonitrogenous, the High ME diet had a higher ME:CP ratio compared to the Low ME diet (13.70 kcal/g vs 12.35 kcal/g). Therefore, birds fed the High ME diet could have overconsumed ME to meet their CP requirement. In the current study, the *ad libitum* fed birds were paired with feed restricted birds, thus feed intake differences between *ad libitum* fed birds fed the High or the Low ME were also imposed on the feed restricted birds.

BW-corrected breast muscle ($P = 0.028$) and liver weight ($P = 0.002$) were higher in birds fed the Low ME diet compared to birds fed the High ME diet (Table 3.4). BW-corrected fat pad weight was higher in birds fed the High ME diet ($P = 0.014$). However, feed intake treatment did not affect any of the BW-corrected carcass components weights. Carcass crude fat percentage was higher in birds fed the High ME diet compared to birds fed the Low ME diet (8.8 % vs 7.1%, $P < 0.001$; Table 3.5), and crude fat percentages increased gradually with increasing feed intake ($P < 0.001$). BW-corrected fat pad weight was the same for all feed intake treatments, therefore the increase in crude fat retention could have occurred in other body tissues. Overall, bird performance was consistent with the literature investigating differences in dietary energy and feed restriction (Leeson et al., 1996; Swennen et al., 2004).

3.4.2 Energy partitioning and net energy

The non-linear mixed model underestimated HP by 13.4% and overestimated RE by 22.8% compared to the CST (Table 3.6). Nonetheless, neither method detected differences in HP and RE between the Low and High ME diet. Figure 3.1 and Figure 3.2 show the relationship between the model methodology and the CST in determining individual measurements for HP and RE. The model estimated the NE_g of the feed 31.5% higher for the Low ME diet and 19.5% higher for the High ME diet compared to the CST (Table 3.7). The NE_g values estimated with the CST were on average 615 kcal/kg lower compared to results from Fraps and Carlyle (1939) and Fraps (1946). Fraps (1946) found an average NE_g value of 1,938 kcal/kg using the CST for an all mash grower diet. NE_g values calculated from reported feed intake and RE from a more recent publication from Wu et al. (2018) ranged from 1,258 to 1,407 kcal/kg in three different experiments, which is 83 to 232 kcal/kg higher than, but similar to, the current results. Wu et al. (2018) used Ross 308 broilers, the same strain as the current experiment. It needs to be considered that since 1946 (Fraps, 1946) broilers have been bred intensively for growth and efficiency and feed ingredients have changed over the years which may have affected the biological energetic efficiency of broilers. For example, residual ME_m (a measurement of biological energetic efficiency) was lower in a 1978

broiler strain than in a 1957 strain (Zuidhof et al., 2014). Thus, it would have been expected that the NE_g content of the diet would have increased as broilers became more efficient. However, previous studies only used *ad libitum* fed birds to determine NE_g content of the diet, whereas our current study used several levels of feed intake. Even though the NE_g calculation corrected for individual feed intake, feed restriction reduced the NE_g value of the feed (Table 3.7). The reason for this could be that a higher proportion of ME goes towards maintenance when gain is constrained. Following this reasoning, environmental factors limiting feed intake therefore decrease NE_g of the feed.

This is the first time that a non-linear mixed model was evaluated against the CST for calculating NE_g values for diets. Comparing the current results to the literature is challenging as many authors do not properly define or calculate the NE_g value of feeds. In some literature, NE_g has also been defined as NE_g plus the energy requirement for maintenance of the body in healthy, fasting, non-reproductive, non-moving, and thermal neutral state (NE for maintenance (**NE_m**), basal metabolic rate, or fasting heat production), divided by the amount of feed consumed (Carré et al., 2014; Noblet et al., 2015; Wu et al., 2018). Or otherwise stated, NE_g is the ME minus the heat increment, where heat increment is the heat produced in excess of NE_m. However, NE_m is affected by animal and environmental factors (Liu et al., 2017). In addition, it is very resource intensive to define NE_m and practically not relevant to measure. More of interest is the effect of the diet on ME_m, which varies with feeding level, environmental temperature, activity, immune status, and any other factor that could affect HP above NE_m. In the current experiment, the requirement for ME_m was 145.10 kcal/$BW^{0.83}$. Considering a BW range from 0.5 to 1.5 kg (82 to 203 kcal), the estimate for ME_m is similar to estimations in the literature of 81 to 187 kcal (Noblet et al., 2015), but lower compared others of 117 to 266 kcal (Zuidhof et al., 2014). However, for higher BW (1.5 to 3.0 kg), the current estimates of ME_m (258 to 361 kcal) were higher compared to estimates of 214 to 304 kcal (Noblet et al., 2015), but still lower than estimates of 330 to 447 kcal (Zuidhof et al., 2014). The current non-linear mixed model may have partitioned ME not completely accurately to HP and RE. The model may have partitioned energy used for gain, but lost as heat, towards the second part of the equation, $1.09 \times BW^{-0.18} \times ADG^{1.19}$, as this energy is required to establish gain. However, energy used for product formation is theoretically included in the portion of ME_m (Zuidhof, 2019). Further studies are needed to improve the current model, potentially providing a solution to the above described issue. Figure 3.3 shows the average ME

requirement per gram of gain as a function of BW and average daily gain. There was a decrease in the ME requirement per gram of gain with increasing BW, especially at low levels of gain. This is contrast with Romero et al. (2009), who found an increment in the ME requirement for gain at greater BW in adult broiler breeders. It is hypothesized that either 1) the efficiency of gain increased in juvenile birds with increased BW or that 2) juvenile birds predominantly deposited lean tissue at very low gain and high BW in the current situation of severe feed restriction. The energy density of lean issue is lower than fat, because protein has a lower energy content than fat (5.5 vs 9.2 kcal/g) and because lean tissue contains about 75% water (Leeson and Summers, 2001). As Hadinia et al. (2018) calculated, the energy requirement per g of lean tissue is approximately 1.38 kcal. This is consistent with the current estimates in Figure 3.3 at high BW and low gain. In addition, birds with low gain had decreased carcass crude fat content and increased moisture content compared to *ad libitum* fed birds and similar CP content (Table 3.5), hence the relative deposition of lean tissue would have been higher in the most feed restricted treatment. Consistent with Romero et al. (2009), ME requirement of gain increased with greater gains, likely because the relative deposition of fat increased, resulting in an increase in the ME requirement of gain.

3.4.3 Heat increment of feeding

To investigate diet-specific HIF, the slopes of the linear regression of individual HP on ME intake of both diets were compared. Figure 3.4 shows the relationship between the ME intake and HP, and the regression lines for the Low ME and High ME diet did not differ (intercept $P = 0.23$; slope $P = 0.24$). As HIF did not differ significantly between diets, the difference in slope was omitted in the final analysis. The HIF was estimated at 26% of the ME intake. This is consistent with results from Swennen (2004), who found that the HIF did not differ between isoenergetic diets with low protein (12.6% CP, 10.6% fat) or low fat (24.4% CP; 0.4% fat) content. The HIF for those diets was estimated between 20 and 23% of the ME intake. Geraert et al. (1990) found HIF to be between 15.9% and 20.9% of the ME intake for diets differing in protein content and they also concluded that HIF did not significantly differ between the diets. Koh and Macleod (1999) found a wider range of the HIF between 7.3% and 35.9% of ME intake depending on ambient temperature, but they did not report diet composition. Although the method of determining HIF in the previously mentioned studies (indirect calorimetry in respiratory cells) differed largely from the current methodology (using a mathematical approach), the outcomes of the current modeling methodology are in the same range.

As mentioned earlier, it was suggested that HIF would be higher for diets with a low ME:CP ratio at higher levels of intake. Overconsumption of CP over ME could result in deamination of excess amino acids releasing heat and an energy source for the bird (Musharaf and Latshaw, 1999; Gous and Morris, 2005). However, the difference in dietary energy or protein content may have not been large enough to detect such an effect.

3.4.4 Heat dissipation

Broilers can manage heat loss via thermoregulatory physiological responses. The temperature of the shank was used as an indicator of the control of heat transfer to their environment through conduction, radiation, and convection (Richards, 1971). There was a significant positive relationship between the temperature of the shank and the energy intake 6, 12, 24, or 48 prior to the measurement, which varied between 0.85°C/100 kcal and 2.74°C/100 kcal (Table 3.8). This indicated that increased energy intake resulted in an increment in shank temperature. Zhou and Yamamoto (1997) found that shank temperature increased with 0.26°C/100 kcal. The difference between results from Zhou and Yamamoto (1997) and the current result may originate from the difference in study design; Zhou and Yamamoto (1997) used short term feed restriction on 49 d to 70 d old *ad libitum* fed broilers, whereas the current study used longer term feed restriction at a younger age. Birds fed the Low ME diet had on average a 0.72°C lower shank temperature compared to birds fed the High ME diet (Table 3.8). It could be possible that birds regulated the heat loss through regulating blood flow through the skin on their shank (Richards, 1971), where birds fed the Low ME diet were losing relatively less heat compared to birds fed the High ME diet. The differences in shank temperature may have also been related to bird fatness, because of the insulative properties of fat in the body skin. Skin fat accounts for 60% of total body fat of *ad libitum* fed broiler chickens and 6.1% to 7.5% of the total BW (Ferrini et al., 2008). In addition, birds fed diets with lower GE:CP ratios (14 to 18 kcal/g) had a reduced hypodermis thickness compared to birds fed diets with a higher GE:CP ratio (16 to 20 kcal/g), which was linked to a decreased adipose tissue deposition in the skin (Kafri et al., 1986). In the current experiment, the Low ME birds had less fat as a percentage of their BW compared to High ME birds (Table 3.5). Birds fed the Low ME diet, with a lower ME:CP ratio, may have had to reduce heat loss through their shanks to compensate for the relative higher heat loss through the body skin compared to birds fed the High ME diet. However, it is unclear how quantitative feed restriction affects percentage of skin fat relative to total body fat or abdominal fat in broilers.

The shank temperature also varied with age, although the environmental temperature did so as well. The environmental temperature at d 22, d 28, d 35, and d 42 was 26.3°C, 21.8°C, 21.8°C, and 21.2°C, respectively. Heat loss rate is depending on the difference between the skin temperature and the environmental temperature (Yahav et al., 2005). It is therefore suggested that the increased shank temperature at d 22 was a result of the higher environmental temperature and unrelated to animal factors.

In the current experiment HP may have also been affected by physical activity of the birds. Although no observational data for behaviour was obtained, frequency of station visits is related to at least one type of physical activity. There was a significant effect of the feed intake treatment on the frequency of station visits (Table 3.9). Birds that were more severely restricted, visited the feeding station more often than *ad libitum* fed birds. This could indicate that the motivation to visit the feeding station was higher in the feed restricted birds compared to the *ad libitum* fed birds. However, this could also have resulted in an increase in HP of the feed restricted birds compared to the *ad libitum* fed birds, as HP increases with increased physical activity (MacLeod et al., 1982, 1988). It was shown that the energy expenditure for locomotion activities in laying hens is about 20 to 25% of HP, and that the total energy expenditure increased by about 53 to 65% when moving at a speed of 1-2 km/h (Kampen, 1976). Also, the increased rate of HP during the light period compared to the dark period associated with standing was estimated to be about 18% of daily HP at 31.12 kcal/d per hen (Li et al., 1991). Therefore, it is hypothesized that feed restricted birds could have had an increased energy expenditure for physical activity which decreased the availability of energy for gain, in addition to the limitation of available nutrients as a direct result of the feed restriction. Research in broilers has also shown that resting energy expenditure is higher during the photoperiod compared to the scotoperiod (Kim et al., 2014), where it was estimated that HP in the photoperiod was 15.80 kcal/d and HP in the scotoperiod was 7.59 kcal/d for each broiler. As birds on the 50% restricted treatment visited the PF system on average 3.9 times during the scotoperiod, whereas the *ad libitum* fed birds only visited the station 0.4 times during the scotoperiod (data not shown), restricted fed birds could have had an increased time of activity during the scotophase and therefore a decrease in the time resting at a low rate of HP in comparison to the *ad libitum* fed birds.

The effect of feed restriction on visit frequency depended on the diet treatment. Whereas *ad libitum* birds fed the Low ME and High ME diet did not differ, the 50% restricted birds fed the

High ME diet visited the feeding station 9 more times per day compared to the 50% restricted birds fed the Low ME diet. This may be related to the link between meal size and number of meals and the physical property of the two feeds. Meal size was 0.7 g smaller and birds had 1.6 meals more per day in the High ME treatment compared to the Low ME treatment (Table 3.9). From observation, the pellets of the High ME diet had a lower quality compared to the Low ME diet, which resulted in more fines. It is known that broilers fed a mash need more time to eat than broilers fed pellets (Nir et al., 1994). As the PF station had a set amount of time per meal, the meal size could therefore have been reduced, requiring birds fed the High ME diet to visit the PF system more often. Alternatively, the High ME diet could also have been less palatable, or the intake of the High ME diet resulted more immediately in signaling of endocrinological satiety mechanisms and therefore a slower rate of intake.

There was no effect of diet or ME intake on the number of peripheral leukocytes or the heterophil to lymphocyte (H:L) ratio (Table 3.10). High H:L ratios are used as an indicator of chronic stress and the number of leukocytes provides one indicator of systemic immune status (More Bayona et al., 2017). Higher H:L ratios have been observed in restricted birds when compared to *ad libitum* fed birds in some studies (Maxwell et al., 1992; Hocking et al., 1993, 1996; Savory et al., 1993), but not in other studies (van Niekerk et al., 1988; Katanbaf et al., 1989; Maxwell et al., 1990; Savory et al., 1996; Jong et al., 2002). The H:L ratios in the current study are higher compared to results from Maxwell (1992). These authors found that at d 42 the H:L ratio was 0.53 for *ad libitum* and 0.76 for feed restricted birds, due to a significant change in the proportion of lymphocytes (57.0% and 47.4% for *ad libitum* and feed restricted birds, respectively). The difference in H:L ratio between the current results and results from Maxwell (1992) could originate from differences in strain, rearing conditions, or health status, but this could also indicate that all birds in the current study were under chronic stress. In recent years, more attention has been paid to strategies that can take advantage of nutrition to modulate the immune system due to the prohibition of feed-added antibiotics in some regions of the world (Korver, 2012). It is still to be defined what proportion of the ME intake is partitioned to maintain and develop the innate and acquired immune system in healthy poultry. Klasing (2007) indicated that at maintenance a young broiler uses about 0.5% of the body's lysine for leukocytes, antibodies and accessory proteins, and that the resting immune system utilizes about 1.2% of the lysine intake in a healthy growing broiler chick. In addition, a difference in the immune system between feed

restricted and *ad libitum* birds may also originate from differences in the responsiveness of leukocytes (More Bayona et al., 2017). In this regard, an assessment of immune function would provide a deeper understanding of immune changes due to feed restriction. Thus, it is recommended that further research studies the maintenance requirements and energetic costs of the immune system in poultry.

3.5 Conclusions

The non-linear model provided a non-invasive real-time method to measure HP and RE in broilers. However, the model was not able to distinguish the NE_g values of the two diets. Estimates of the NE_g values decreased when feed intake was reduced. The HIF could be determined with the modeling methodology and was in the range of values in the literature. Additional measurements on heat dissipation, physical activity, and immune status indicated that the energetic content of the diet and feed restriction affect some parameters (shank temperature, feeding station visits), but not others (leukocyte counts, H:L ratio, and immune cell function). Further research is needed to understand dietary factors affecting ME available for productive processes, including more comprehensive analysis on the energy expenditure on activity and immunity.

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3.8 Tables

Table 3.1 Ingredient and nutritional composition of the starter (d 0 to d 14) and grower (d 15 to d 35) diets fed to broilers in the current experiment.

	Starter	Low ME grower	High ME grower
Ingredient composition, g/kg			
Corn, ground	75.00	179.68	180.07
Wheat, ground	317.37	444.21	377.64
Soybean meal (48% CP)	175.00	289.49	310.12
Faba beans, ground	80.00	-	-
Wheat cracks, ground	80.00	-	-
Wheat, whole (14.5% CP)	75.00	-	-
Meat and bone meal	67.00	-	-
Canola meal	50.00	-	-
Canola, whole	40.00	-	-
Animal fat	22.00	-	-
Canola oil		22.51	68.30
Limestone	5.00	10.12	9.92
MHA[1]	2.70	-	-
Salt, NaCl	2.60	3.57	3.64
Dicalcium Phosphate	-	15.17	15.44
L-Lysine HCL	1.80	0.44	-
Enzyme[2]	1.00	-	-
Poultry trace mineral premix[3]	1.00	-	-
Broiler vitamin premix[3]	1.00	-	-
Broiler grower premix[4]	-	4.99	5.00
Choline liquid 70%	0.85	-	-
Choline chloride premix[5]	-	4.99	5.00
DL-Methionine	-	1.29	1.36
L-Threonine	0.70	0.07	-
Bacitracin MD	0.50	-	-
Monensin premix 20%	0.50	-	-
Coban®	-	0.50	0.51
Vitamin E 5000 IU/kg	-	3.00	3.00
25-OH Vitamin D_3	0.40	-	-
Copper sulfate	0.40	-	-
Ethoxyquin, 66%	0.18	-	-
Celite	-	19.96	20.01
Calculated composition, as fed basis			
MEn, kcal/kg	3,073	2,900	3,150
CP, %	23.16	22.00	22.00

Lys, %	1.25	1.12	1.12
PCD[6] Lys, %	1.10	0.96	0.96
PCD Met, %	0.51	0.41	0.42
PCD Met + Cys, %	0.83	0.73	0.73
Analyzed composition, as fed basis			
Dry Matter	87.8	87.3	86.1
ME, kcal/kg	-	3,111	3,383
CP, %	25.7	25.2	24.7
Fat, %	7.5	3.9	7.9

[1] Methionine hydroxy analogue: 84% Ca salt of 2-hydroxy-4-(methylthio)butanoic acid, Novus International, Inc., St. Charles, MO.

[2] Avizyme 1302 feed enzyme for use in poultry diets containing at least 20% wheat (Danisco Animal Nutrition, Marlborough, Wiltshire, UK).

[3] Combined poultry trace mineral premix and broiler vitamin premix contributed per kg of diet: vitamin A, 10,000 IU; vitamin D3, 4,000 IU; vitamin E, 50 IU; vitamin K3, 3.1 mg; riboflavin, 10 mg; thiamine, 2 mg; pyridoxine, 5 mg; vitamin B12, 0.02 mg; niacin, 65 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.2 mg; iron, 80 mg; copper, 15 mg; manganese, 110 mg; zinc, 100 mg; iodine, 2 mg; selenium, 0.3 mg.

[4] Contributed per kg of diet: vitamin A, 10,000 IU; vitamin D3, 4,000 IU; vitamin E, 50 IU; vitamin K3, 4 mg; riboflavin, 10 mg; thiamine, 4 mg; pyridoxine, 5 mg; vitamin B12, 0.02 mg; niacin, 65 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.2 mg; iron, 80 mg; copper, 20 mg; manganese, 120 mg; zinc, 100 mg; iodine, 1.65 mg; selenium, 0.3 mg; choline, 2.64 mg.

[5] Contributed per kilogram of diet 400 mg/kg choline.

[6] Pre-cecal digestible.

Table 3.2 Regression coefficients of the nonlinear model[1] estimating daily ME intake as a function of BW and average daily gain.

Parameter	Estimate	SEM	t-value	P > t
a	145.00	8.48	17.10	< 0.001
b	0.83	0.04	19.06	< 0.001
c	1.09	0.37	2.97	0.005
d	-0.18	0.05	-3.75	< 0.001
e	1.19	0.07	17.07	< 0.001
V	399.39	32.69	12.22	< 0.001
Vu	151.41	44.79	3.38	0.001

[1] Equation: $MEI_d = (a + u)\ BW^b + 1.09 \times BW^d \times ADG^e$ $MEI_d \sim N(\mu,V)$, $u \sim N(0, V_u)$, where MEI_d = daily ME intake (kcal/d), BW = body weight (kg), and ADG = average daily gain (g/d). Bayesian information criterion = 3422.

Table 3.3 BW at d 45 (BW), cumulative feed intake (CFI), total ME intake (MEI), cumulative gain (Gain), and feed conversion ratio (FCR) of broilers fed either a Low ME (3,111 kcal/kg) or a High ME (3,383 kcal/kg) diet from d 14 to d 45. Birds were pair-fed through a precision feeding system with lead birds eating *ad libitum* (100%) and followers were allowed to eat either 50%, 60%, 70%, 80%, or 90% of the paired lead's cumulative feed intake.

Effect	Diet	Feed intake	BW (g)	SEM	CFI (g)	SEM	MEI (kcal)	SEM	Gain (g)	SEM	FCR	SEM
Diet	Low ME		2,280	36.2	3,099[a]	38.6	9,641[b]	126	1,881	34.6	1.659	0.0168
	High ME		2,261	35.2	2,988[b]	37.6	10,108[a]	123	1,863	33.7	1.616	0.0163
Feed intake		50 %	1,639[f]	65.2	2,071[f]	69.6	6,721[f]	228	1,227[f]	62.3	1.694	0.0302
		60 %	1,843[e]	65.2	2,444[e]	69.6	7,924[e]	228	1,468[e]	62.3	1.666	0.0302
		70 %	2,156[d]	60.4	2,870[d]	64.5	9,310[d]	211	1,749[d]	57.7	1.643	0.0279
		80 %	2,414[c]	65.2	3,247[c]	69.6	10,531[c]	228	2,013[c]	62.3	1.619	0.0302
		90 %	2,675[b]	60.4	3,622[b]	64.5	11,751[b]	211	2,280[b]	57.7	1.593	0.0279
		100 %	2,896[a]	54.0	4,008[a]	57.7	13,012[a]	189	2,494[a]	51.6	1.609	0.0250
Source of variation[2]			P - value									
Diet			0.70		0.047		0.012		0.70		0.07	
Feed intake			< 0.001		< 0.001		< 0.001		< 0.001		0.16	
Diet × Feed intake			0.92		0.97		0.75		0.95		0.70	

[a-f] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$).

Table 3.4 Individual BW-corrected breast, fat pad, liver, legs without skin, heart, gastro-intestinal tract (GIT), and empty GIT weight of broilers fed either a Low ME (3,111 kcal/kg) or High ME (3,383 kcal/kg) diet from d 14 to d 45. Birds were pair-fed through a precision feeding system with lead birds eating *ad libitum* (100%) and followers were allowed to eat either 50%, 60%, 70%, 80%, or 90% of the paired lead's cumulative feed intake.

Effect	Diet	Feed intake	Breast (g)	SEM	Fat pad (g)	SEM	Liver (g)	SEM	Legs (g)	SEM	Heart (g)	SEM	GIT (g)	SEM	Empty GIT (g)	SEM
Diet	Low ME		477[a]	6.3	14.2[b]	1.9	43.5[a]	0.85	475	6.5	9.7	0.5	181	8.4	119	3.4
	High ME		457[b]	6.3	21.0[a]	1.9	39.6[b]	0.84	478	6.5	10.3	0.5	167	8.4	120	3.4
Feed intake		50 %	415	22.7	21.3	6.9	46.2	3.04	467	23.3	9.2	1.8	230	30.3	146	12.2
		60 %	432	17.8	17.7	5.4	43.6	2.39	462	18.3	9.0	1.4	205	23.7	127	9.6
		70 %	451	11.4	18.7	3.5	41.1	1.53	479	11.7	10.3	0.9	182	15.2	117	6.1
		80 %	478	11.5	14.1	3.5	41.6	1.55	474	11.9	10.4	0.9	173	15.4	120	6.2
		90 %	506	14.6	20.5	4.4	38.2	1.97	475	15.0	11.2	1.1	142	19.5	110	7.9
		100 %	521	19.1	13.4	5.8	38.6	2.57	499	19.7	9.9	1.5	114	25.6	96	10.3
Covariable			BW (g/kg)													
BW			162	29.0	23.8	8.8	25.2	3.90	187	29.8	4.3	2.3	125	38.7	56	15.6
Source of variation			P - value													
Diet			0.028		0.014		0.002		0.73		0.42		0.26		0.78	
Feed intake			0.16		0.60		0.51		0.69		0.72		0.36		0.16	
Diet × Feed intake			0.82		0.91		0.36		0.92		0.51		0.39		0.44	
BW			< 0.001		0.011		< 0.001		< 0.001		0.063		0.003		0.001	

[a,b] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$).

Table 3.5 Carcass crude protein (CP), crude fat (CF), ash, and moisture as percentage of BW at d 45 of broilers fed either a Low ME (3,111 kcal/kg) or a High ME (3,383 kcal/kg) diet from d 14 to d 45. Birds were pair-fed through a precision feeding system with lead birds eating *ad libitum* (100%) and followers were allowed to eat either 50%, 60%, 70%, 80%, or 90% of the paired lead's cumulative feed intake

Effect	Diet	Feed intake	CP (%)	SEM	CF (%)	SEM	Ash (%)	SEM	Moisture (%)	SEM
Diet	Low ME		20.5	0.13	7.1^{b}	0.26	3.1	0.05	70.2^{a}	0.34
	High ME		20.8	0.12	8.8^{a}	0.26	3.1	0.04	68.1^{b}	0.33
Feed intake		50 %	20.6	0.23	5.7^{c}	0.47	3.2	0.08	71.4^{a}	0.61
		60 %	20.6	0.23	6.9^{bc}	0.47	3.2	0.07	69.9^{ab}	0.61
		70 %	20.7	0.21	7.8^{b}	0.44	3.1	0.08	69.5^{b}	0.57
		80 %	20.7	0.23	7.6^{b}	0.47	3.2	0.08	69.4^{b}	0.61
		90 %	20.6	0.21	9.6^{a}	0.44	3.0	0.07	67.6^{c}	0.57
		100 %	20.6	0.19	10.1^{a}	0.39	2.9	0.06	67.1^{c}	0.51
Source of variation			P - value							
Diet				0.072		< 0.001		0.529		< 0.001
Feed intake				0.995		< 0.001		0.077		< 0.001
Diet × Feed intake				0.50		0.76		0.47		0.83

[a-c] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$).

Table 3.6 Heat production (HP) and retained energy (RE) for broilers fed either a Low ME (3,111 kcal/kg) or a High ME (3,383 kcal/kg) diet from d 14 to d 45 as calculated with the comparative slaughter technique (CST) or a mathematical non-linear model (model[1]) and the difference between the model and the CST method (Δ).

	HP						RE					
Diet	CST	SEM	model	SEM	Δ	SEM	CST	SEM	model	SEM	Δ	SEM
	kcal											
Low ME	6,264	234	5,301	217	-963[b]	68	3,606	265	4,659	261	1,053[a]	67
High ME	6,387	229	5,659	213	-728[a]	67	3,951	259	4,620	256	669[b]	66
Source of variation	P - value											
Diet	0.71		0.24		0.018		0.36		0.91		< 0.001	

[1] The estimated equation was $MEI_d = (145.10 + u)\ BW^{0.83} + 1.09 \times BW^{-0.18} \times ADG^{1.19}$ and $u \sim N(0, V_u)$, $MEI_d \sim N(\mu,V)$, where MEI_d = daily ME intake (kcal/d), BW = body weight (kg), and ADG = average daily gain (g/d). The error term *u* was associated with each bird, variance parameters *V* and *Vu* were estimated in the regressions. The first part of the equation, $(145.10 + u) \times BW^{0.83}$, represented HP, the second part of the equation ($1.09 \times BW^{-0.18} \times ADG^{1.19}$) represented RE. Estimated HP and RE per period were summed to reflect total HP and total RE over the experimental period (d 14 to d 45).

Table 3.7 Net energy for gain (NE_g) value of the feed for broilers fed either a Low ME (3,111 kcal/kg) or a High ME (3,383 kcal/kg) diet from d 14 to d 45 as calculated with the comparative slaughter technique (CST) or a mathematical non-linear model (model[1]) and the difference between the model and the CST method (Δ). Birds were pair-fed through a precision feeding system with lead birds eating *ad libitum* (100%) and followers were allowed to eat either 50%, 60%, 70%, 80%, or 90% of the paired lead's cumulative feed intake

Effect	Diet	Feed intake	NE_g model	SEM	NE_g CST	SEM	ΔNE_g	SEM
			kcal/kg					
Diet	Low ME		1,448	18.5	1,101[b]	22.5	346[a]	20.1
	High ME		1,493	18.0	1,249[a]	22.0	244[b]	19.6
Feed intake		50 %	1,367[d]	33.4	996[c]	40.6	371[a]	36.3
		60 %	1,423[cd]	33.4	1,099[bc]	40.6	324[ab]	36.3
		70 %	1,454[bcd]	30.9	1,163[b]	37.6	291[abc]	33.6
		80 %	1,500[abc]	33.4	1,169[b]	40.6	331[ab]	36.3
		90 %	1,543[a]	30.9	1,307[a]	37.6	237[bc]	33.6
		100 %	1,534[ab]	27.6	1,317[a]	33.6	216[c]	30.1
Source of variation			P - value					
Diet			0.09		< 0.001		0.001	
Feed intake			0.002		< 0.001		0.020	
Diet x Feed intake			0.82		0.87		0.78	

[1] The estimated equation was $MEI_d = (145.10 + u)\ BW^{0.83} + 1.09 \times BW^{-0.18} \times ADG^{1.19}$ and $u \sim N(0, V_u)$, $MEI_d \sim N(\mu, V)$, where MEI_d = daily ME intake (kcal/d), BW = body weight (kg), and ADG = average daily gain (g/d). The error term *u* was associated with each bird, variance parameters *V* and *Vu* were estimated in the regressions. The first part of the equation, $(145.10 + u) \times BW^{0.83}$, represented HP, the second part of the equation ($1.09 \times BW^{-0.18} \times ADG^{1.19}$) represented RE. Estimated HP and RE per period were summed to reflect total HP and total RE over the experimental period (d 14 to d 45).

[a-d] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$).

Table 3.8 Temperature of the surface of the shank of broilers fed either a Low ME diet (3,111 kcal/kg) or a High ME diet (3,383 kcal/kg) from d 14 to d 45, measured at 22, 28, 45, or 42 days of age, and analyzed with a covariate for ME intake during the 6, 12, 24, or 48 hours prior to the temperature measurement.

			Covariate 6 h		Covariate 12 h		Covariate 24 h		Covariate 48 h	
Effect	Diet	Age	Temperature	SEM	Temperature	SEM	Temperature	SEM	Temperature	SEM
			°C							
Diet	Low ME		35.53[b]	0.25	35.66[b]	0.24	35.65[b]	0.23	35.67[b]	0.22
	High ME		36.45[a]	0.25	36.33[a]	0.24	36.33[a]	0.23	36.32[a]	0.22
Age		22 d	38.55[a]	0.37	39.12[a]	0.36	39.04[a]	0.34	39.60[a]	0.35
		28 d	33.42[c]	0.36	33.33[c]	0.34	33.56[c]	0.32	33.67[c]	0.32
		35 d	36.33[b]	0.36	36.02[b]	0.34	35.78[b]	0.33	35.57[b]	0.33
		42 d	35.67[b]	0.37	35.50[b]	0.35	35.58[b]	0.33	35.12[b]	0.33
Covariate			°C/100 kcal							
ME intake			2.74	0.50	2.34	0.30	1.56	0.17	0.85	0.09
Source of variation			P - value							
Diet			0.011		0.050		0.035		0.042	
Age			< 0.001		< 0.001		< 0.001		< 0.001	
Diet x Age			0.21		0.42		0.41		0.31	
ME intake			< 0.001		< 0.001		< 0.001		< 0.001	

[a-c] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$).

Table 3.9 Number of times birds accessed the feeding station (visits), number of daily meals, daily meal to visit (M:V) ratio, and meal size for broilers fed either a Low ME diet (3,111 kcal/kg) or a High ME (3,383 kcal/kg) from d 14 to d 45[1]. Birds were pair-fed through a precision feeding system with lead birds eating *ad libitum* (100%) and followers were allowed to eat either 50%, 60%, 70%, 80%, or 90% of the paired lead's cumulative feed intake.

Effect	Diet	Feed intake	Visits (#)	SEM	Meals (#)	SEM	M:V ratio (%)	SEM	Meal size (g)	SEM
Diet	Low ME		61.7	1.12	14.3^{b}	0.20	43.8^{b}	0.96	8.4^{a}	0.08
	High ME		61.6	1.06	15.9^{a}	0.19	46.9^{a}	0.91	7.7^{b}	0.07
Feed intake		50 %	122.3^{a}	2.01	8.8^{e}	0.36	8.9^{f}	1.72	9.1^{a}	0.14
		60 %	86.1^{b}	1.98	10.5^{d}	0.36	20.0^{e}	1.70	9.1^{a}	0.14
		70 %	57.4^{c}	1.84	13.4^{c}	0.33	27.9^{d}	1.58	8.0^{b}	0.13
		80 %	49.1^{d}	2.01	17.6^{b}	0.36	49.0^{c}	1.72	7.3^{cd}	0.14
		90 %	33.2^{e}	1.84	18.5^{b}	0.33	66.6^{b}	1.58	7.7^{bc}	0.13
		100 %	21.8^{f}	1.64	21.7^{a}	0.30	99.5^{a}	1.40	7.0^{d}	0.11
Diet × Feed intake	Low ME	50 %	117.8^{b}	3.09	8.8^{g}	0.56	10.4^{gh}	2.65	9.1^{ab}	0.21
		60 %	91.8^{c}	2.62	10.0^{fg}	0.47	16.2^{g}	2.25	9.5^{a}	0.18
		70 %	63.8^{e}	2.62	13.2^{e}	0.47	25.1^{ef}	2.25	8.2^{c}	0.18
		80 %	42.0^{g}	3.09	17.8^{c}	0.56	54.4^{c}	2.65	7.4^{de}	0.21
		90 %	34.4^{gh}	2.62	15.7^{d}	0.47	57.0^{c}	2.25	8.8^{b}	0.18
		100 %	20.4^{i}	2.33	20.3^{b}	0.42	99.6^{a}	2.00	7.3^{e}	0.16
	High ME	50 %	126.8^{a}	2.57	8.8^{g}	0.46	7.5^{h}	2.21	9.0^{ab}	0.18
		60 %	80.4^{d}	2.97	11.0^{f}	0.54	23.8^{f}	2.55	8.8^{b}	0.20
		70 %	50.9^{f}	2.57	13.6^{e}	0.46	30.7^{e}	2.21	7.8^{cd}	0.18
		80 %	56.3^{f}	2.57	17.5^{c}	0.46	43.6^{d}	2.21	7.3^{de}	0.18
		90 %	32.0^{h}	2.57	21.3^{b}	0.46	76.1^{b}	2.21	6.5^{f}	0.18
		100 %	23.1^{i}	2.30	23.0^{a}	0.42	99.4^{a}	1.97	6.7^{f}	0.16
Source of variation			P - value							
Diet			0.93		< 0.001		0.021		< 0.001	
Feed intake			< 0.001		< 0.001		< 0.001		< 0.001	

Diet × Feed intake	< 0.001	< 0.001	< 0.001	< 0.001
Age[2]	0.07	< 0.001	< 0.001	< 0.001
Age × Diet	0.14	0.33	0.91	1.00
Age × Feed intake	0.98	0.93	0.96	1.00
Age × Diet × Feed intake	1.00	1.00	0.95	1.00

[a-i] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$).

[1]Data till the end of d 44 was included as birds were euthanized at d 45.

[2]Number of meals decreased with age from 17.5 at d 20 to 11.8 at d 44; M:V ratio decreased with age from 59.7% at d 20 to 39.2% at d 44; meal size increased with age from 4.3g at d 20 to 11.5g at d 44.

Table 3.10 Number of total leukocytes and heterophil, lymphocyte, combined monocyte and macrophage percentages of total leukocyte number, and heterophil to lymphocyte (H/L) ratio in blood samples taken at d 45 of birds fed Low (3,111 kcal/kg) or High (3,383 kcal/kg) ME diets from d 14 to d 45.

Diet	Total Leukocytes (#)	SEM	Heterophils (%)	SEM	Lymphocytes (%)	SEM	Monocytes and Macrophages (%)	SEM	H/L ratio	SEM
Low ME	32,197	2100	43.5	3.3	40.9	4.3	15.6	2.8	1.2	0.2
High ME	28,049	1817	39.2	3.1	44.3	4.0	17.0	2.6	1.0	0.2
Source of variation	P - value									
Diet	0.16		0.37		0.58		0.73		0.54	
ME intake[1]	0.31		0.46		0.98		0.29		0.88	

[1] P - values for the covariable ME intake was not significant, therefore the regression coefficient was not shown. Mean ME intake was 9,641±126 kcal for the Low ME diet and 10,108±123 kcal for the High ME diet over the experimental period.

3.9 Figures

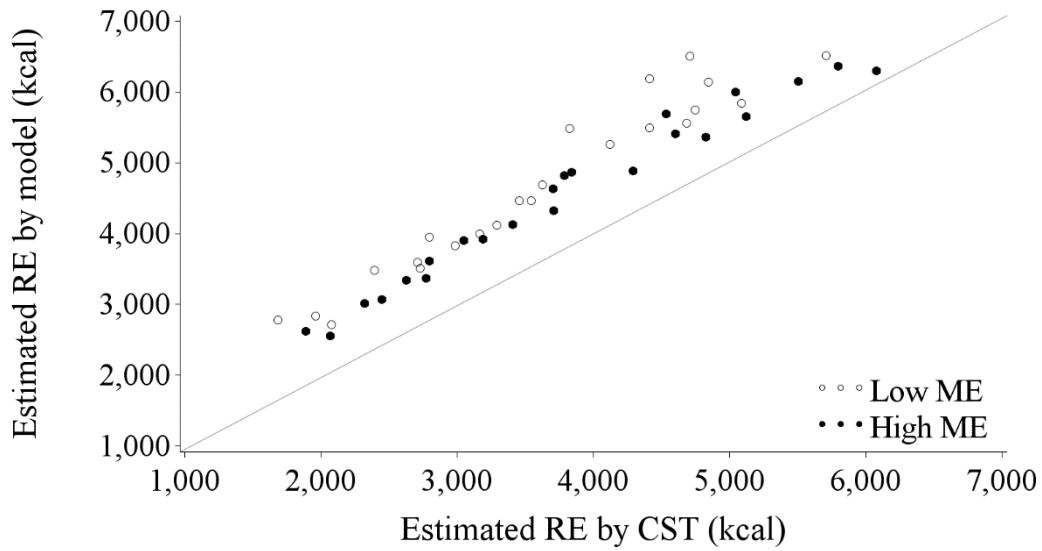

Figure 3.1 Retained energy (RE) estimated by a non-linear equation explaining ME intake as a function of metabolic BW and gain (model) compared to the RE estimated by the comparative slaughter technique (CST) of broilers fed either fed a Low ME (3,111 kcal/kg) or High ME (3,383 kcal/kg) diet from d 14 to d 45, where the model overestimated RE. The solid grey line indicates where the model would have estimated the same value as the CST.

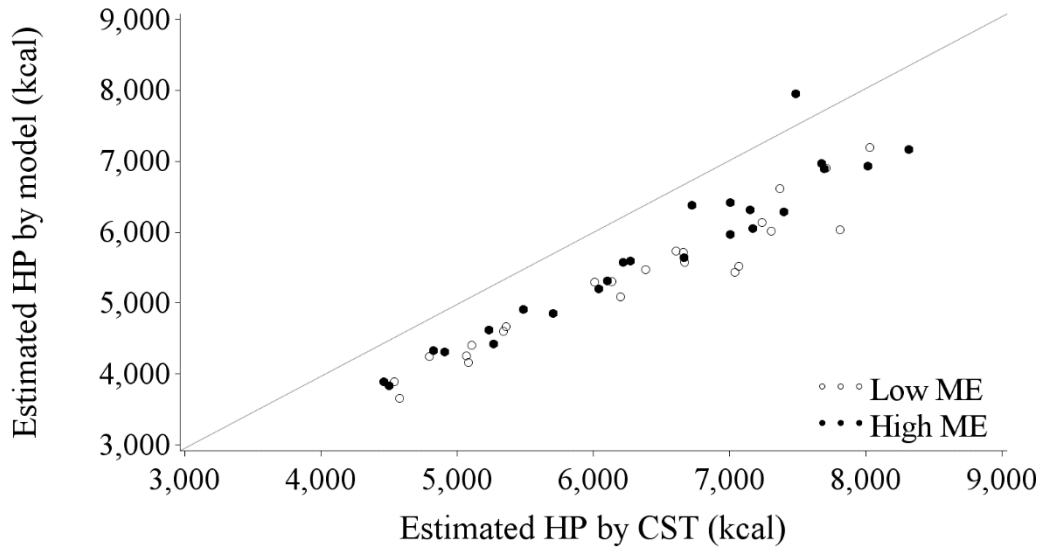

Figure 3.2 Total heat production (HP) estimated by a non-linear equation explaining ME intake as a function of metabolic BW and gain (model) vs calculated through the comparative slaughter technique (CST) of broilers fed either fed a Low ME (3,111 kcal/kg) or High ME (3,383 kcal/kg) diet from d 14 to d 45, where the model underestimated HP. The solid grey line indicates where the model would have estimated the same value as the CST.

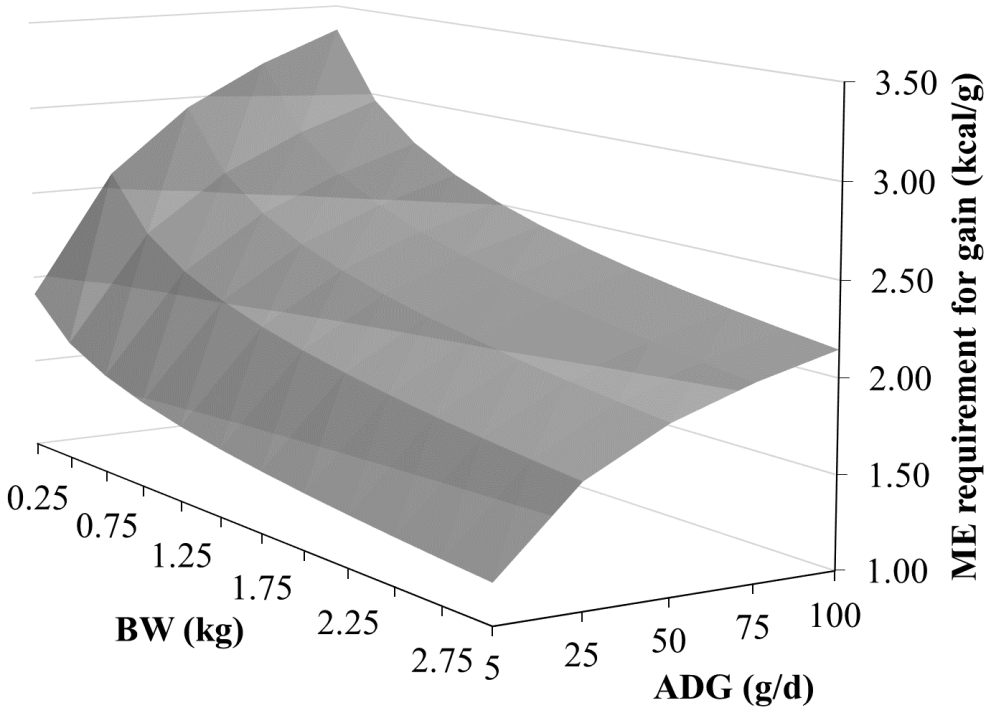

Figure 3.3 ME requirement per gram of average daily gain (ADG), as a function of body weight (BW) and ADG, as predicted by a non-linear model explaining ME intake as a function of metabolic BW and gain of broilers from d 14 to d 45. The estimated equation was $MEI_d = (145.10 + u)\ BW^{0.83} + 1.09 \times BW^{-0.18} \times ADG^{1.19}$ and $u \sim N(0, V_u)$, $MEI_d \sim N(\mu,V)$, where MEI_d = daily ME intake (kcal/d), BW = body weight (kg), and ADG = average daily gain (g/d). The error term u was associated with each bird. The second part of the equation ($1.09 \times BW^{-0.18} \times ADG^{1.19}$) represented retained energy (gain) per day.

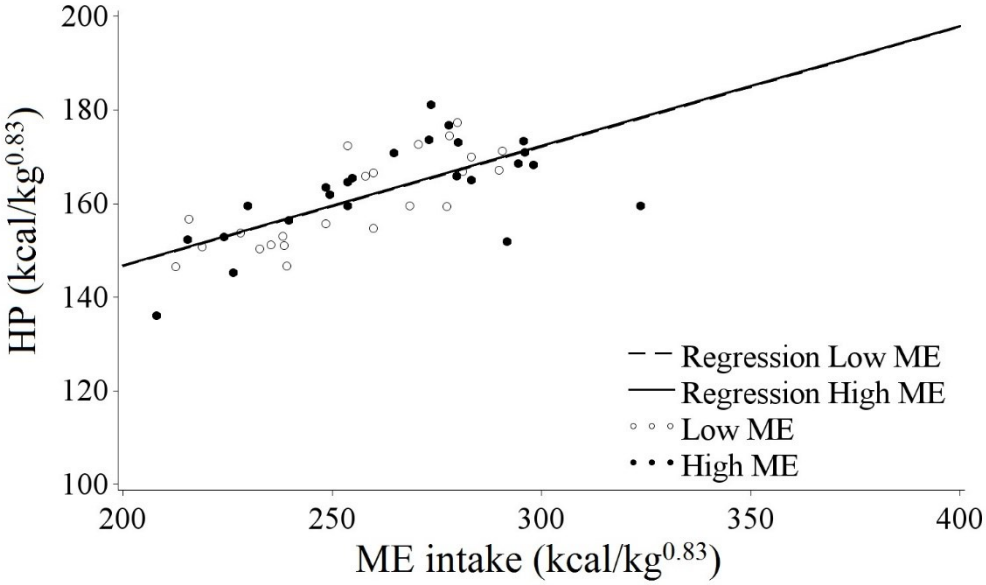

Figure 3.4 Linear regression of heat production (HP) and average daily ME intake (ME intake) per unit of metabolic BW ($kg^{0.83}$) as estimated by the comparative slaughter technique of broilers fed either fed a Low ME (3,111 kcal/kg) or High ME (3,383 kcal/kg) diet from d 14 to d 45. Linear regression equations were: HP = 95.64 kcal + 0.26 × ME intake for the Low ME diet and HP = 95.44 kcal + 0.26 × ME intake for the High ME diet.

CHAPTER 4.

Early photostimulation at the recommended body weight reduced broiler breeder performance[3]

4.1 Abstract

To synchronize the onset of sexual maturity in the face of high BW variation, the age at photostimulation has been increasing in the broiler breeder industry. This experiment studied the effects of increased BW and earlier photostimulation on broiler breeder reproductive performance where within-treatment BW uniformity was very high. The experiment tested BW and age at photostimulation treatments in a 2 x 2 factorial arrangement. Hens (n = 120) were fed with a precision feeding system to allocate feed individually following the breeder-recommended target BW (Standard) or to a 22% heavier target BW curve reaching the Standard 21 wk BW at 18 wk (High). Hens were photostimulated at either 18 wk (18WK) or 21 wk (21WK) with a 16L:8D photoschedule. Age at first egg (AFE) and individual egg production to 55 wk were recorded. Differences were reported as significant if $P \leq 0.05$. The AFE was decreased and maturation interval between photostimulation and AFE was shorter for hens on the High BW treatment compared to the Standard BW treatment (178.1 vs. 194.7 d and 41.8 vs 58.2 d, respectively). Hens on the 21WK treatment had a decreased AFE compared to the 18WK treatment (177.0 d vs. 195.9 d) and their maturation interval was shorter (30.0 d vs. 69.9 d). The CV for AFE was higher in the 18WK treatment compared to the 21WK treatment (28.2% vs. 11.2%). Total egg production was higher for hens on the High BW treatment compared to the Standard BW treatment (129.4 vs 92.8, respectively). Total egg production was higher for hens on the 21WK treatment compared to the 18WK treatment (138.4 vs 83.8, respectively). Egg weight of Standard BW x 18WK hens was lower compared to High BW x 18WK hens. Current recommended breeder BW may be too low for optimal sexual maturation after photostimulation. It is concluded that even when BW variation is minimized, photostimulation at 18 wk of age is not recommended.

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4.2 Introduction

Broiler breeders are kept on a strict level of feed restriction to manage their reproductive performance. Every year the level of feed restriction becomes more severe, as genetic growth potential of broilers increases while the recommended broiler breeder BW profiles are not adjusted (Renema et al., 2007a). Especially during the rearing period when broiler breeders are most restricted, high competition for feed within broiler breeder flocks results in high BW variation. It is known that pullets that are underweight at photostimulation subsequently exhibit lower egg production (Robinson and Robinson, 1991; Melnychuk et al., 2004). Flocks with a high variation in BW exhibit low production efficiency as a high proportion of hens weigh less than the target at photostimulation. Therefore, it was previously recommended to delay the moment of photostimulation to 22 or 23 wk (Robinson et al., 1996; Renema et al., 2001a, 2007b). More recently Pishnamazi et al. (2014) concluded that the beneficial effects of later photostimulation were only BW dependent, which would mean that accelerated growth could facilitate earlier photostimulation. Minimizing BW variation in hens photostimulated at wk 23 did not advance age at sexual maturity nor increase egg production (Romero et al., 2009a), yet it is unclear if at earlier photostimulation the same effect could be expected. If earlier photostimulation results in earlier age at sexual maturity, this would shorten the rearing period. If there would be no negative effects on settable egg production, shortening the rearing period would be economically beneficial as this shortens the period of no return or lengthen the productive period for hatching egg producers.

Several studies have suggested that there is a minimum age and a minimum BW for the ability to respond to photostimulation (photosensitivity) and sexually mature (Katanbaf et al., 1989; Lewis et al., 2007a). Lewis (2007a) showed that the minimum age after which broiler breeders can be photosensitive is 10 wk. Before this age, the onset of lay does not advance when hens are photostimulated and hens respond as if they are maintained on long days from hatch. After wk 24 broiler breeder pullets respond uniformly to photostimulation, irrespective of genetic line or feeding program (Melnychuk et al., 2004), indicating that all pullets have dissipated their photorefractory state. Accelerating growth advances the dissipation of the photorefractory state, so increasing target BW will result in earlier photosensitivity (Lewis et al., 2007b). As genetic selection for growth traits did not change time between photostimulation and age at first egg (Pishnamazi et al., 2014), advancing the age at photostimulation could be feasible if BW variation were to be controlled. Yuan et al. (1994) concluded that increased BW can facilitate advancing the

onset of lay with earlier photostimulation, however they noted that increased feed allowance would have to be continued during the laying period, to support the increased maintenance requirements of higher BW hens.

As recent developments in feeding technology have allowed group housed hens to be reared towards individual target BW with less than 2% CV for BW (Zuidhof et al., 2016, 2017), the aim of this research was to investigate the effects of BW and age at photostimulation on broiler breeder reproductive performance in group housed hens, when within-treatment variation in BW is minimized. It was hypothesized that hens following a higher BW profile would show faster dissipation of photorefractoriness at the same age at photostimulation and therefore show an increased egg production, due to a lengthened laying period because of an earlier onset of lay.

4.3 Material and methods

4.3.1 Experimental design

The animal protocol for the study was approved by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care Guidelines and Policies (CCAC, 2009). The experiment was conducted as a 2 x 2 factorial arrangement of treatments with pullets being reared following the breeder-recommended target BW curve (Aviagen, 2016; Standard), or an accelerated target BW curve reaching the 21 wk BW at 18 wk (High), and photostimulated at either wk 18 (18WK) or wk 21 (21WK). As a result, the High target BW was 22% heavier than the Standard target BW at 21 wk of age. As birds were individually fed to achieve the defined BW treatments, each individual bird was considered to be one experimental unit.

4.3.2 Animals and housing

The experimental protocol was similar to that previously described by van der Klein (2017). In brief, Ross 708 broiler breeder chicks were provided by Aviagen (Huntsville, Alabama, USA; n = 120) and were randomly allocated to one of 4 environmentally controlled rooms (30 chicks per room). Each room was equipped with a precision feeding (PF) station (Zuidhof et al., 2016, 2017), which controlled individual feed intake to achieve and adhere to the assigned target BW curves. Water was provided *ad libitum* during the entire experiment. From day 0 to 16, birds were trained to use the PF station and fed *ad libitum*. At d 16, birds were 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 and were

allowed access to feed for a duration of 45 s when birds qualified to eat. Birds qualified to eat when their BW as measured by the PF station was lower than their treatment target BW. When their measured BW was equal to or higher than their treatment target, birds were ejected from the station and not provided access to feed. At the start of the experiment, pairs of rooms were randomly assigned to either a 18WK or 21WK photostimulation treatment. For the first 2 days, a 23L:1D photoschedule was used after which the light period was decreased by 2 h daily until 8L:16D and remained constant until photostimulation. Photostimulation was achieved in a single step to 16L:8D. The light source used was a 60% red, 20% green, and 20% blue LED light bulb (PGR-11, AgriLux, Cambridge, ON) set to provide 8 lux during the rearing phase and 25 lux during the production phase. For the first 3 wk, chicks received a standard wheat based starter diet (2,900 AME, 19% CP, 1.1% Ca). From wk 4 to 2 wk after photostimulation, pullets received a wheat and barley based grower diet (2,589 AME, 14.2% CP, and 0.9% Ca). From 2 wk after photostimulation to wk 34, hens received a wheat based peak layer diet (2,689 AME, 15.0% CP, and 3.3% Ca). From wk 35 to wk 55, hens received a wheat based post peak layer diet (2,682 AME 14.6% CP, and 3.3% Ca).

At wk 18 a nest box equipped with Radio Frequency Identification (RFID) readers was installed in each room, which assigned eggs to individual hens. The day before photostimulation 3 roosters were added per room. Roosters were reared in a separate location under an 8L:16D photoschedule and fed towards the recommended target BW curve (Aviagen, 2016) using a PF station.

4.3.3 Data collection

A detailed description of data collection methods can be found in van der Klein (2017). In brief, the PF station recorded BW and feed intake individually on a per visit basis after individual feeding started. Because it would not be possible for floor eggs to be linked with individual hens, and hens on different BW treatments were housed in the same room, all hens were palpated daily via the cloaca to detect hard-shelled eggs in the shell gland. This was essential to measure age at first egg (AFE) and individual egg production from wk 20 to wk 36. As the majority of the birds on the 21WK treatment had entered lay by wk 36, from 36 wk onward, daily palpation was performed every second wk. Eggs laid in the RFID-equipped nest boxes that could be traced to specific hens were weighed daily. Eggs between 40 g and 90 g were included in statistical analysis for egg weight. Eggs weighing more than 90 g were considered double-yolked eggs and they were

analyzed separately. The incidence of mortality (including culls) was recorded throughout the experiment. At wk 55, 16 hens per BW x photostimulation treatment were killed by cervical dislocation directly after lights turned on and dissected. The abdominal fat pad, full gastro-intestinal tract (GIT), breast muscle (total weight of pectoralis major and pectoralis minor), heart, liver, oviduct (without content), and ovary weight were recorded. In addition, the number of yellow follicles larger than 10 mm (LYF) was recorded.

4.3.4 Statistical analysis

All ANOVA were conducted using the MIXED procedure of SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012). Pairwise differences between means were determined with the PDIFF option of the LSMEANS statement and were considered significant at $P \leq 0.05$. Tukey's range test was used to compare treatment means. Hen was the experimental unit, except for cumulative hens in lay and percentage of hens that did not commence egg production before wk 55. For the latter, hens within each BW treatment within each chamber were randomly assigned to one of 3 groups, after which the parameters were calculated per group and group was used as experimental unit. The model used for the CV for BW, egg production, cumulative hens in lay, rate of lay, and egg weight data included BW treatment, age at photostimulation, and age as fixed effects and all 2- and 3-way interactions. Additional analysis for egg weight included BW at AFE as a covariate within the statistical model. Random variation due to hen was accounted for in all serial measurements. Rate of lay was calculated as the hen day egg production of those hens that had reached their AFE. Due to insufficient data points prior to 30 wk of age, egg weight was analyzed from wk 30 onward. The model used for cumulative feed intake (CFI), AFE, maturation interval, percentage of hens that did not commence egg production before wk 55, CV for AFE, CV for BW at AFE, and cumulative egg production data included BW treatment and age at photostimulation as fixed effects, and their interaction. Dissection data were reported as percentage of live BW to correct for BW variation within the BW treatment. The model used for the dissection data included BW treatment and age at photostimulation as fixed effects, and their interaction and a binary random effect, whether or not the hen had laid her first egg.

4.4 Results and discussion

4.4.1 BW, BW variation, and cumulative feed intake

Actual Standard and High BW profiles closely matched their target profiles up to 24 wk of age (Figure 4.1). The CV in BW throughout the experiment is reported in Table 4.1. According to the analysis of variance, the CV in BW was dependent on age ($P = 0.012$) and on BW treatment ($P < 0.001$). As no significant pair-wise differences were indicated after Tukey's range test to compare LSMeans, Table 4.1 shows result of the least significant difference test. At both wk 18 and wk 21 the CV in BW in all treatments was less than 1%, which confirmed that the PF stations were able to minimize variation in BW at photostimulation. All High BW hens had reached the 21 wk breeder recommended target BW at wk 18. At wk 20, the BW of High BW hens was higher than Standard BW hens ($P < 0.001$) and there was no effect of age at photostimulation on BW. At wk 20, the High BW hens were 2,423 and 2,417 g, and Standard BW hens were 1,978 and 1,975 g (± 21 g) on the 18WK and 21WK treatments, respectively. The CV in BW of the High BW treatment increased after photostimulation compared to the Standard BW treatment. As previously reported by van der Klein et al. (2017; Chapter 5), the High BW treatment hens started laying earlier compared to hens on the Standard BW treatment and some High BW treatment hens sexually matured at a BW below their target BW (Table 4.3 and Figure 4.1). Some of the earlier laying hens remained at a BW lower than their target throughout the study, increasing the BW variability in the High BW treatment. The interaction between effect of age at photostimulation and BW on CFI was not significant during the rearing phase ($P = 0.181$), which means that there was no difference in CFI between hens reared towards the 21 wk BW at 18 wk or at 21 wk. As CFI was calculated from d 16 to photostimulation during rearing, CFI was lower in the 18WK compared to 21WK treatment (Table 4.2). During rearing, CFI was lower in the Standard treatment compared to the High BW treatment ($P < 0.001$), due to lower ME requirements for growth and maintenance. Although CFI during the laying phase was calculated over a 3-week longer period for the 18WK treatment, CFI was 2,349 g lower in the 18WK treatment compared to the 21WK treatment. Presumably, the lower egg production in the 18WK treatments (see section below) compared to the 21WK treatment and the associated lower ME requirements for egg production accounted for this difference (Romero et al., 2009b). Mortality from d 16 to the end of the trial averaged 7.95%, and did not differ significantly between treatment groups (data not shown).

4.4.2 Onset of sexual maturity and egg production

Results for the onset of sexual maturity are reported in Table 4.3. As expected, there was a lower AFE for the High BW treatment compared to the Standard BW treatment (178.1 d vs. 194.7 d, P = 0.036). This is in line with the conclusion in previous literature that heavier broiler breeders mature earlier compared to lighter weight birds (Lewis et al., 2005, 2007b; Lewis and Morris, 2005; Lewis and Gous, 2006). AFE was not different for the High BW x 18WK birds compared to the Standard BW x 21WK birds (182.8 d vs 180.4 d), where it was expected that the High BW x 18WK treatment would have matured earlier, as it was anticipated that they would have reached the minimum BW target for sexual maturation. AFE of the 21WK treatment was similar to the most recent report of broiler breeders photostimulated at 21 wk (Pishnamazi et al., 2014), where hens in the current study matured at 177.0 d and in the previously mentioned study at 179.5 d. Renema et al. (2007b) found an interaction between BW and age at photostimulation. They found that when photostimulation occurred at 18 wk of age, hens with a BW 25% below the recommended target at wk 12 came into production 17.4 d after hens with a BW 200% of the recommended target at wk 12. However, when they delayed photostimulation until wk 22, BW profile did not affect the timing of sexual maturation. In the current experiment, no such interaction was found. It is suggested that the larger difference between BW profiles and age at photostimulation, and greater BW variation within treatment in the study by Renema et al. (2007b) increased the ability to detect an interaction compared to the current study.

Looking at the rate at which birds started laying (Figure 4.3), from the rapid increase at wk 22 and the flattening of the curve after wk 24, it can be estimated that approximately 40% of the Standard BW birds, and approximately 60% of the High BW birds were responsive to photostimulation at wk 18. These birds responded uniformly to photostimulation by sexually maturing, thus indicating that all 3 levels of the reproductive axis (hypothalamus, pituitary and ovary) were in a ready state. Hens that came into production after this point matured spontaneously and not uniformly suggesting that one or more component of the axis was not responsive at the time of photostimulation. Comparing this to the responsiveness to photostimulation of birds on the 21WK treatment, approximately 90% of the birds were responsive to photostimulation, irrespective of BW treatment. The observation that not all High BW birds had dissipated their photorefractory state at wk 18 could be explained in two ways. First, it could indicate that the breeder recommended 21-wk BW target (Aviagen, 2016) was below the actual required minimum

BW for onset of sexual maturity after photostimulation. Second, it could indicate that some hens at wk 18 had not reached the age required to sexually mature, irrespective of their BW. For both factors, BW and age, it is hypothesized that there is most likely not a fixed threshold but rather a mean with some level of variation around it. This was previously concluded by Lewis et al. (2007), however, none of the described trials in their paper entailed a comparison with the exact same BW at two different photostimulation ages. In addition, studies in the past have always dealt with high CV for BW. A model proposed by Lewis et al. (2007b) predicting the age at 50% production in broiler breeders given a single increment in photoperiod from BW at wk 20, did not accurately estimate the current results. Only for the High BW x 21WK treatment their model estimated the mean age at 50% production close to the current result with an estimated mean of 195.4 d, compared to the current calculated mean of 194.3. Estimated means of the other treatments were over 22 days lower than current calculated means. In addition, estimated CV of age at 50% production did not compare with the current results. Therefore, it was concluded that the current results do not fit the models as described by Lewis et al. (2007b). All hens on the High BW x 21WK treatment laid their first egg before the end of the experiment, but 11.7% of the hens on the High BW x 18WK treatment never commenced egg production (Table 4.3). For the Standard BW hens, 31.9% and 3.3% never commenced egg production on the 18WK and 21WK treatment, respectively. It is hypothesized that hens which never commenced egg production either did not meet their individual required BW for sexual maturation, or were missing a different metabolic incentive to sexually mature, such as a sharp increase in feed intake (discussed later). Lewis et al. (2007b) acknowledged that faster growth increased the rate of dissipation of juvenile photorefractoriness. Although not analyzed in the current experiment, body composition may have played an additional role. Over the past decades abdominal fat pad weight as a percentage of BW has been decreasing from 4.9 ± 0.2% in a 1978 selected line to 2.9 ± 0.2% in a 2015 selected line at 21 wk of age (Reimer et al., 2017). This is hypothesized to be related to the delay in AFE in modern breeder lines, as Lewis et al. (2003) previously showed that hens from a leaner male breeder line had a delayed sexual maturity compared to a female breeder line. In addition, there is a growing body of literature in human medicine that describes that an early onset of sexual maturity coincides with an increased body fat percentage in women (Walvoord, 2010). De Beer and Coon (2007) concluded that total lean protein mass was a threshold for the onset of sexual maturity. However, dissection results at the end of the current experiment did not indicate a difference in

the proportion of breast muscle between birds on the Standard and High BW treatment. Still, hens on the Standard BW treatment had lower proportional fat pad weight compared to hens on the High BW treatment (Table 5, 1.6% vs. 2.2%, $P < 0.018$). In addition, birds that had commenced egg production before wk 55 had a 3.2% (of BW) greater proportion of breast muscle compared to birds that had not commenced egg production before wk 55, while proportion of fat pad tended to be smaller ($P = 0.083$). Logistical constraints on bird numbers did not allow us to assess body composition around sexual maturation, hence, a direct relationship between body composition and AFE in the current experiment could not be studied. However, the observations at wk 55 indicate that a required fat threshold mass may be critical for the onset of lay. The novel feeding method the current study used may have altered body composition, as pullets were fed multiple times a day in small meals, instead of one meal every one or two days. This could have changed both their total and proportional lean and fat tissue mass, and therefore affected reproductive performance, as compared to conventional feeding methods (Carneiro, 2016). Further studies are needed to reveal the extent to which this is the case. In addition, as previously mentioned, every year breeding companies have been recommending similar BW profiles for broiler breeders while increasing growth potential of broilers (Renema et al., 2007a). Data from the current experiment support the statement that breeding companies have now approached the limit of the ability of broiler breeders to reach their mature BW within the recommended BW profiles, therefore it is hypothesized that the current BW recommendations from the primary breeder are too low.

Hen day egg production as observed in Figure 2, is a combination of the number of hens that are in production and the rate of lay of the individual hens. To illustrate this, a 50% hen day egg production could mean that 50% of the hens are in production and laying 100% of the days. Alternatively, it could mean that 100% of the hens are in production, but they only lay at a 50% rate. To separate these two interpretations of the same parameter, the rate of lay was calculated in the current study as the hen day egg production for the subgroup of hens that were in production, i.e. had reached AFE. There was a significant effect of BW treatment, photostimulation treatment, and age on rate of lay (for all effects $P < 0.001$). Mean rate of lay was 69.9 ± 0.8% and 59.9 ± 1.0% for High and Standard BW treatment, and 61.3 ± 1.1% and 68.4 ± 0.8% for the 18WK and 21WK treatment, respectively. The rate of lay is assumed to be associated with laying sequence analysis, as both reproductive parameters give an indication on the reproductive performance of hens that have laid their first egg. Prime sequence length and mean sequence length are positively

related to increased rate of lay. Previously, it was found that an increase in BW profile during the rearing phase reduced prime sequence and mean sequence length, which would mean a reduced rate of lay (Zuidhof et al., 2007), which is in contrast with the current results. Also in contrast with the current results, it was found that there was no effect of age at photostimulation on laying sequence traits (Joseph et al., 2002; Zuidhof et al., 2007).

The maturation interval was increased in the 18WK treatment compared to the 21WK treatment (69.9 vs. 30.0 d, Table 4.3). Robinson et al. (1996) reported maturation intervals between 50.6 to 24.2 d, when broiler breeders were photostimulated at ages ranging from 120 to 160 d. Renema et al. (2007b) reported maturation intervals of 41.5 d and 29.9 d for the hens photostimulated at wk 18 or wk 22, respectively. The increased maturation interval in the current results could have been the result of genetic changes over the years, however, Pishnamazi et al. (2014) concluded that genetic selection for growth traits did not change the maturation interval. They reported maturation intervals of 49.2, 41.2, 32.5, and 24.0 d for hens photostimulated at 17, 19, 21, and 23 wk of age, respectively. The maturation interval for the 21WK treatment in the current study is comparable to these results, but the maturation interval of the 18WK treatment is much larger. Previously, hypotheses were proposed that the rate of sexual maturation after photostimulation increases, when photostimulation occurred later, such that for every day that photostimulation was delayed, AFE was delayed between 0.21 d to 0.40 d (Yuan et al., 1994; Robinson et al., 1996; Renema et al., 2001b; Joseph et al., 2002; Ciacciariello and Gous, 2005; Pishnamazi et al., 2014). However, in the current study, AFE was advanced by 19 d when photostimulation was delayed by 21 d, resulting in an advance of 0.90 d for every day that photostimulation was delayed. The counterintuitive result that delaying photostimulation actually advanced the AFE could be related to the alternative feeding method in the current study. As previously mentioned, the PF station provided a reduced meal size and an increased frequency of meals time separated over the day as compared to conventional feeding methods. In addition, there was an altered feed allocation strategy after photostimulation. As compared to conventional methods, there was no overall increase in feed allocation on the flock level, only once an individual hen had laid her first egg, there was a production-related feed increase for this individual, as losing the weight of the egg resulted in access to feed. This is important, as feeding program just before and right after photostimulation can affect AFE (Melnychuk et al., 2004; Ciacciariello and Gous, 2005; Renema et al., 2007b). Melnychuk et al. (2004) compared broiler breeders that were either

restricted fed or *ad libitum* fed after photostimulation. They showed that the maturation interval was shorter for *ad libitum* fed birds compared to restricted birds photostimulated at wk 21 (36.4 vs. 49.9 d, respectively), but that there was no difference in maturation interval for the restricted and full-fed birds photostimulated at wk 24 (28.2 d). At photostimulation, BW in the 21WK treatment was higher compared to the 18WK treatment and birds were fed more in the 21WK treatment to sustain their growth and maintenance requirements. The higher feed allocation could possibly also have provided a stimulus for contributed to incentive to start sexual maturation. Therefore, in addition to the possibility of not having reached the minimum BW or the required body composition, the absence of the metabolic signal related to feed intake could have increased maturation interval as compared to previous research (Robinson et al., 1996; Renema et al., 2007b).

The CV for AFE was higher in birds photostimulated at wk 18 compared to birds photostimulated at wk 21 (28.2% vs. 11.2%, P = 0.025, Table 4.3). Renema et al. (2001a) reported a CV for AFE of 5.02% and 4.02% for 19 wk and 21 wk photostimulated broiler breeders, respectively. Pishnamazi et al. (2014) also showed no significant difference in SD of age at sexual maturity for hens photostimulated at 17, 19, 21 or 23 wk. However, in line with the current results, Robinson et al. (1996) reported that birds photostimulated at the older ages (up to 23 wk) reached sexual maturity with less variation for BW at first egg and in AFE. In addition, the novel LED light source could have affected dissipation of the photorefractory state. Bédécarrats et al. (2016) hypothesized that the hypothalamus, receives both metabolic cues and cues from photoreceptors to dissipate the photorefractory state, and that light from the red spectrum is required for hypothalamic stimulation (Mobarkey et al., 2010). The current trial used an LED light source that included 60% red, 20% green, and 20% blue light, as compared to conventionally used incandescent light, which mostly consists of red spectrum light. However, it was previously found that there was no difference in AFE between hens reared under 60% red or 60% green light (Rodriguez, 2017). Therefore, it was not expected that the novel light source would have influenced the current results.

No interaction was found between BW and age at photostimulation for egg production (Figure 4.2). Cumulative egg production was higher in the High BW treatment and 21WK treatment compared to the Standard BW treatment and 18WK treatment, and the effect of photostimulation and BW were independent (Table 4.4). Previously, Robinson et al. (1996)

discussed that total egg production did not differ between hens photostimulated between 120 and 160 d and reared towards the same target BW curve (159.7 eggs until wk 60). Also Joseph et al. (2002) did not find a difference in total egg production between hens photostimulated at wk 21 or wk 23 (131.1 to wk 48). Cumulative egg production until wk 55 for the 21WK treatment was 138.4 eggs, which is comparable to previous reports, however, in the 18WK treatment egg production was decreased to 83.8 eggs. Gibson et al. (2008) reported that total egg production was greater for hens fed every day after photostimulation, compared to hens on a skip-a-day feeding treatment until 8% production (172 vs. 155 to wk 65). This is a further indication, as previously discussed, that the alternative PF method after photostimulation could have had an important influence on total egg production in this study.

4.4.3 Egg weight

Egg weight increased with age for all treatments ($P < 0.001$), independent of BW treatment or age at photostimulation (data not shown). The number of eggs > 90 g was not different between treatments (data not shown). There was an interaction between the effect of BW and age at photostimulation on egg weight ($P < 0.001$). Egg weight was not different between High BW and Standard BW birds on the 21WK treatment, but egg weight of Standard BW x 18WK hens was 3.4 g lower compared to High BW x 18WK hens (Table 4.4). The general understanding is that egg weight is positively correlated with hen weight or hen weight at sexual maturity (McDaniel et al., 1981). However, the difference in hen weight was the same between Standard and High BW hens within the 18WK and 21WK treatments throughout the experiment (Figure 4.1). In addition, after including BW at sexual maturity as a covariate in the egg weight analysis, the interaction between BW and photostimulation remained significant ($P < 0.001$, data not shown). Previously, Pishnamazi et al. (2014) concluded that egg weight differences between hens photostimulated at different ages was attributed solely to the difference in BW at sexual maturity, not by the effect of photostimulation. In other studies, delayed photostimulation resulted in a difference in BW without subsequent differences in egg weight (Yuan et al., 1994; Robinson et al., 1996). Joseph et al. (2002) reported that hens photostimulated at wk 23 laid heavier eggs compared to hens photostimulated at wk 21 (60.8 g vs 59.7 g, respectively), although it was unclear if this difference could be attributed to the higher BW at sexual maturity for hens photostimulated at wk 23 compared to hens photostimulated at 21 wk (3,105 g vs 2,966 g). Possibly BW is not the only factor playing a role in determining egg weight. Another factor could be feeding strategy around time of sexual

maturity, as increased feeding levels during the onset of lay have been reported to result in higher egg weights (Zuidhof et al., 2007). However, in the current study all birds were fed according to a predefined precision feeding strategy. The rate of lay could also have affected egg weight, as hens that lay less eggs may have a higher rate of yolk deposition per follicle, leading to higher egg weight (McLeod et al., 2014; Tůmová et al., 2017). However, this hypothesis is not supported by the current results, as the rate of lay was lower in the Standard BW x 18WK treatment compared to the High x 18WK treatment (55.5% vs. 67.1%, $P < 0.001$), whereas the eggs of the Standard BW x 18WK treatment weighed less compared to the High x 18WK treatment.

4.4.4 Body conformation

Results for body conformation are summarized in Table 4.5. As previously referred to, logistical constraints on bird numbers did not allow us to assess body conformation around sexual maturation. Pishnamazi et al., (2014) reported that differences in body conformation at sexual maturity were only related to BW at sexual maturity. They suggested that the results of previous studies indicating that frame size, fatness, and proportion of breast would be increased by later photostimulation (Renema et al., 2001a, 2007b) could be explained by BW differences alone, without an additional effect of the later photostimulation. The current results show that at wk 55, proportional liver weight and GIT weight increased in the 21WK treatment compared to the 18WK treatment. In addition, fat pad and liver weight as a percentage of live BW were higher in the High BW treatment compared to the Standard BW treatment (2.2% vs 1.6%, and 1.8% vs 1.5%, respectively). It is hypothesized that the increased liver and GIT weight resulted from a higher metabolic rate and increased feed intake to support increased egg production in the 21WK treatment and the High BW treatment.

4.5 Conclusions

Even when within-treatment variation in BW was minimized, decreasing the age at photostimulation from wk 21 to wk 18 increased the variability in age at sexual maturity and decreased reproductive performance of broiler breeders. The current results indicate that the recommended breeder BW at wk 21 is below the optimal target for maturation after photostimulation. It is hypothesized that the hypothalamic responsiveness and dissipation of the photorefractory state might also be influenced by additional metabolic triggers resulting from a difference in feeding frequency and feed allocation.

4.6 Acknowledgements

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4.8 Tables

Table 4.1 Coefficient of variation for BW (BW CV) at various ages of hens fed with a precision feeding station toward a High and Standard BW[1] curve and photostimulated (PS) at wk 18 or wk 21.

			BW CV (%)								
	BW	PS	Wk 4	Wk 7	Wk 14	Wk 18	Wk 21	Wk 27	Wk 40	Wk 54	Pooled SEM
Wk[2]			5.7^{x}	2.4^{yz}	0.3^{z}	0.4^{z}	0.9^{yz}	2.8^{xyz}	3.5^{xy}	3.7^{xy}	1.0
BW x wk	High		7.9^{a}	2.1	0.3	0.4^{a}	0.9	4.4^{a}	5.4^{a}	6.0^{a}	1.1
	Standard		3.4^{b}	2.6	0.4	0.4^{a}	0.8	1.3^{b}	1.6^{b}	1.3^{b}	
PS x wk		18	7.1	2.1	0.4	0.5	0.7	3.0	3.0	4.0	1.3
		21	4.2	2.7	0.3	0.3	1.0	2.7	4.0	3.3	
BW x PS x wk	High	18	11.0^{a}	1.8	0.4	0.5	1.0	4.6	5.5^{a}	7.5^{a}	1.6
		21	4.9^{b}	2.5	0.2	0.3	0.8	4.1	5.4^{ab}	4.6^{ab}	
	Standard	18	3.3^{b}	2.3	0.3	0.5	0.4	1.3	0.5^{b}	0.5^{b}	
		21	3.6^{b}	2.8	0.4	0.4	1.2	1.2	2.6^{ab}	2.0^{b}	

$^{a-c}$ LSMeans within a column and treatment group lacking a common superscript differ ($P \leq 0.05$).
$^{w-z}$ LSMeans within a row lacking a common superscript differ ($P \leq 0.05$).
[1] Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).
[2] P - values for the different sources of variation were as follows: Wk, P = 0.012; BW, P < 0.001; PS, P = 0.686; BW x PS, P = 0.044; Wk x BW, P = 0.014; Wk x PS P = 0.895; Wk x BW x PS, P = 0.448.

Table 4.2 Cumulative feed intake (CFI) of broiler breeder hens from d 16 to photostimulation (Rearing phase) and from photostimulation to wk 55 (Laying phase), fed toward a High and Standard BW[1] curve and photostimulated (PS) at wk 18 or wk 21.

			Rearing phase		Laying phase	
	BW	PS	CFI (g)	SEM	CFI (g)	SEM
BW	High		7,985[a]	38	34,295[a]	700
	Standard		6,510[b]	40	28,004[b]	728
PS		18	6,404[b]	41	29,975[b]	755
		21	8,091[a]	37	32,324[a]	672
BW x PS	High	18	7,105	56	33,939	1,029
		21	8,865	52	34,652	950
	Standard	18	5,704	60	26,011	1,104
		21	7,316	52	29,996	950
Source of variation			P value			
BW			< 0.001		< 0.001	
PS			< 0.001		0.022	
BW x PS			0.181		0.109	

[a-c]LSMeans within a column and treatment group lacking a common superscript differ ($P < 0.05$).

[1]Hens followed either the breeder-recommended target BW curve (Standard) or an accelerated target BW curve reaching the 21 wk BW at 18 wk (High).

Table 4.3 Age at first egg (AFE)[1], maturation interval (MI)[1], percentage of hens that did not commence egg production before 55 wk (Not laid), BW at AFE, coefficient of variation (CV) for AFE[1], and coefficient of variation for BW at AFE[1] of hens fed toward a High and Standard BW[2] curve and photostimulated (PS) at wk 18 or wk 21.

	BW	PS	AFE (d)	SEM	MI (d)	SEM	Not laid (%)	SEM	BW at AFE (g)	SEM	AFE CV (%)	SEM	BW at AFE CV (%)	SEM
BW	High		178.1[b]	5.3	41.8[b]	5.3	5.8	5.15	3,157[a]	54	16.2	3.46	11.7	0.90
	Standard		194.7[a]	5.8	58.2[a]	5.8	17.6		2,824[b]	59	23.2		13.2	
PS		18	195.9[a]	6.1	69.9[a]	6.1	21.8[a]	5.15	3,046	62	28.2[a]	3.46	13.3	0.90
		21	177.0[b]	4.9	30.0[b]	4.9	1.7[b]		2,935	50	11.2[b]		11.6	
BW x PS	High	18	182.8	8.0	56.8	8.0	11.7	7.28	3,176	81	25.2	4.89	14.4[a]	1.27
		21	173.5	6.9	26.5	6.9	0.0		3,137	70	7.2		9.0[b]	
	Standard	18	209.0	9.1	83.0	9.1	31.9		2,915	93	31.2		12.3[ab]	
		21	180.4	7.0	33.4	7.0	3.3		2,732	72	15.1		14.2[a]	
Source of variation			P – value											
BW			0.036		0.036		0.121		< 0.001		0.225		0.290	
PS			0.017		< 0.001		0.012		0.166		0.025		0.237	
BW x PS			0.220		0.220		0.258		0.366		0.853		0.044	

[a-b]LSMeans within a column and treatment group lacking a common superscript differ ($P < 0.05$).

[1] Hens that did not commence egg production before wk 55 were excluded from the analysis.

[2] Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

Table 4.4 Cumulative egg production (Eggs) from wk 23 to wk 55 and mean egg weight of hens fed toward a High and Standard BW[1] curve and photostimulated (PS) at wk 18 or wk 21.

	BW	PS (wk)	Eggs	SEM	Egg weight (g)	SEM
BW	High		129.4[a]	7.3	63.9[a]	0.2
	Standard		92.8[b]	7.3	62.6[b]	0.2
PS		18	83.8[b]	7.5	62.3[b]	0.3
		21	138.4[a]	7.0	64.1[a]	0.2
BW x PS	High	18	106.8	10.5	64.0[a]	0.4
		21	152.0	10.0	63.8[a]	0.2
	Standard	18	60.8	10.8	60.6[b]	0.4
		21	124.7	10.0	64.5[a]	0.2
Source of variation			P – value			
BW			0.001		< 0.001	
PS			< 0.001		< 0.001	
BW x PS			0.368		< 0.001	

[a-c]LSMeans within a column and treatment group lacking a common superscript differ ($P < 0.05$).

[1]Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

Table 4.5 Breast, fat pad, liver, heart, gastro intestinal tract (GIT), ovary and oviduct weight as percentage of live BW, and number of large yellow follicles (LYF) of hens at 55 wk fed toward a High and Standard BW[1] target and photostimulated (PS) at wk 18 or wk 21 and that either commenced egg production (Laid) or did not commence egg production (Not laid) before wk 55[2].

	BW	PS (wk)	Breast (%)	SEM	Fat pad (%)	SEM	Liver (%)	SEM	Heart (%)	SEM	GIT (%)	SEM	Ovary (%)	SEM	Oviduct (%)	SEM	LYF	SEM
BW	High		26.1	0.74	2.2[a]	0.26	1.8[a]	0.09	0.35	0.016	4.5	0.17	1.2	0.18	1.0	0.16	3.8	0.58
	Standard		26.7	0.62	1.6[b]	0.22	1.5[b]	0.08	0.34	0.013	4.6	0.14	0.9	0.15	0.8	0.13	3.0	0.54
PS		18	27.0	0.61	1.7	0.21	1.5[b]	0.08	0.33	0.013	4.4[b]	0.14	1.0	0.15	0.8	0.13	3.2	0.52
		21	25.8	0.76	2.1	0.27	1.8[a]	0.09	0.35	0.016	4.7[a]	0.17	1.2	0.18	1.0	0.16	3.6	0.60
BW x PS	High	18	26.9	0.88	2.0	0.31	1.6	0.11	0.34	0.019	4.3	0.20	1.0	0.21	0.9	0.19	3.1	0.68
		21	25.3	0.95	2.5	0.33	1.9	0.12	0.35	0.020	4.7	0.21	1.5	0.23	1.1	0.21	4.4	0.73
	Standard	18	27.1	0.76	1.5	0.26	1.4	0.10	0.32	0.016	4.4	0.17	0.9	0.18	0.7	0.16	3.3	0.67
		21	26.4	0.91	1.7	0.32	1.7	0.11	0.35	0.019	4.8	0.20	0.9	0.22	0.9	0.20	2.7	0.69
Laid			24.8[b]	0.41	2.3	0.14	2.0[a]	0.05	0.39[a]	0.009	4.7	0.09	1.7[a]	0.10	1.4[a]	0.09	5.6[a]	0.31
Not laid			28.0[a]	1.07	1.6	0.38	1.3[b]	0.14	0.30[b]	0.023	4.3	0.24	0.4[b]	0.26	0.4[b]	0.23	1.2[b]	0.92
Source of variation			P – value															
BW				0.410		0.018		0.021		0.545		0.542		0.134		0.274		0.223
PS				0.155		0.170		0.007		0.156		0.043		0.208		0.259		0.559
BW x PS				0.594		0.568		0.985		0.572		0.953		0.181		0.853		0.104
Laid				0.008		0.083		< 0.001		0.001		0.116		< 0.001		< 0.001		< 0.001

[a-c]LSMeans within a column and treatment group lacking a common superscript differ ($P < 0.05$).

[1] Hens followed either the breeder-recommended target BW curve (Standard) or an accelerated target BW curve reaching the 21 wk BW at 18 wk (High).

[2] The effect of whether hens had commenced egg production or not before wk 55 on body composition did not depend on age at photostimulation.

4.9 Figures

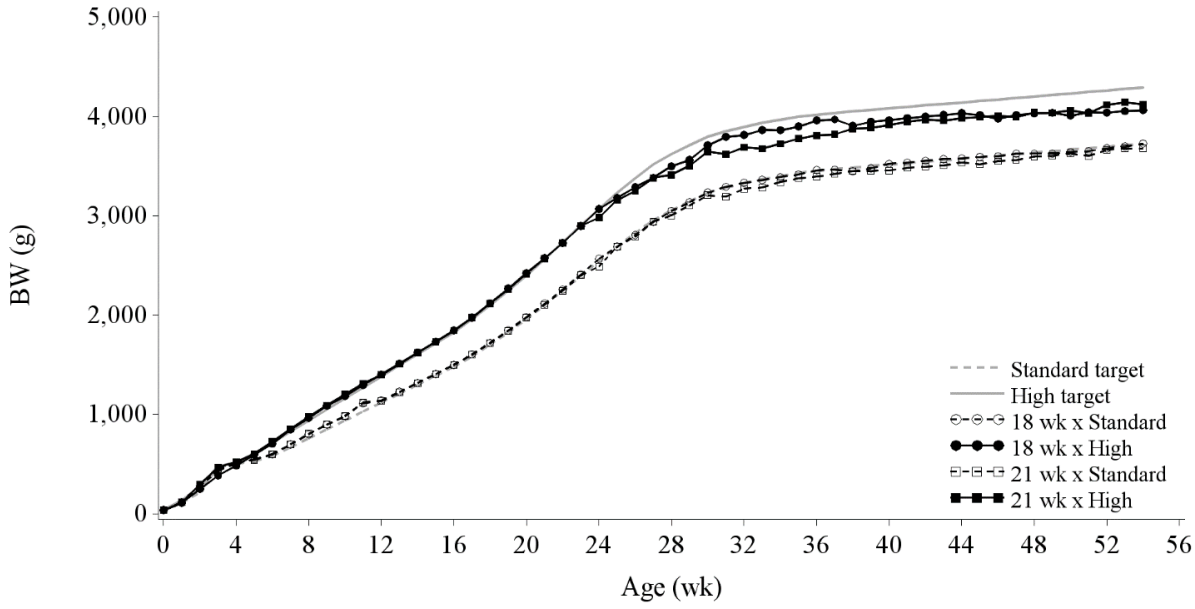

Figure 4.1 BW of hens fed towards either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and photostimulated at wk 18 or wk 21.

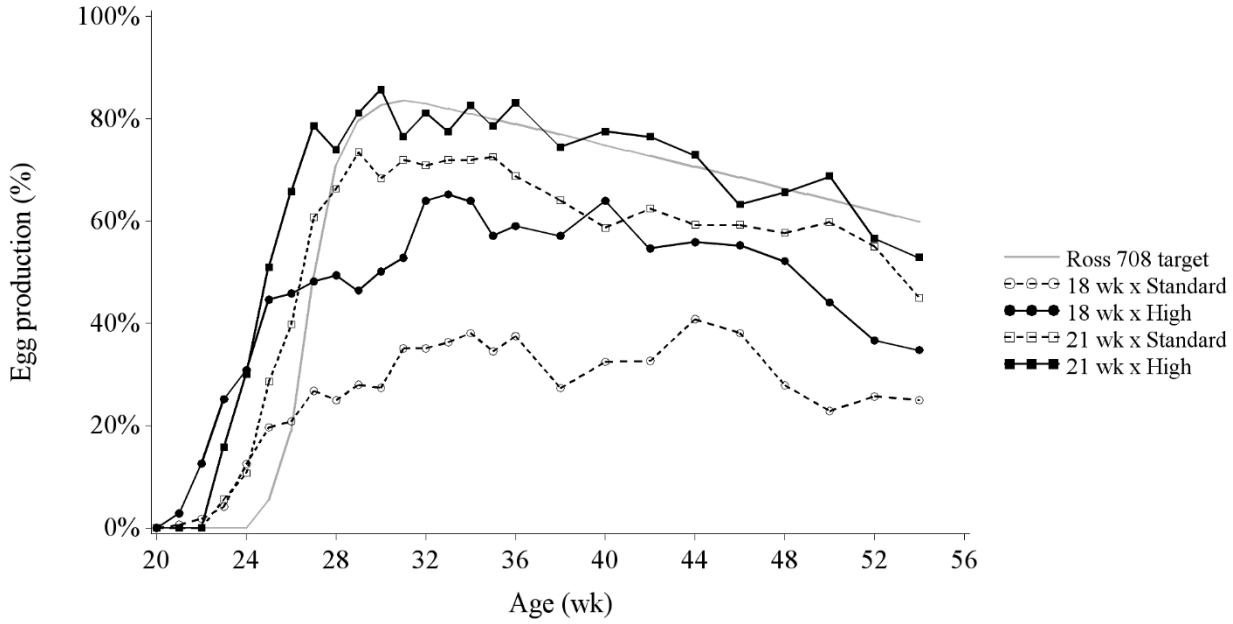

Figure 4.2 Hen day egg production of hens fed towards either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and photostimulated at wk 18 or wk 21.

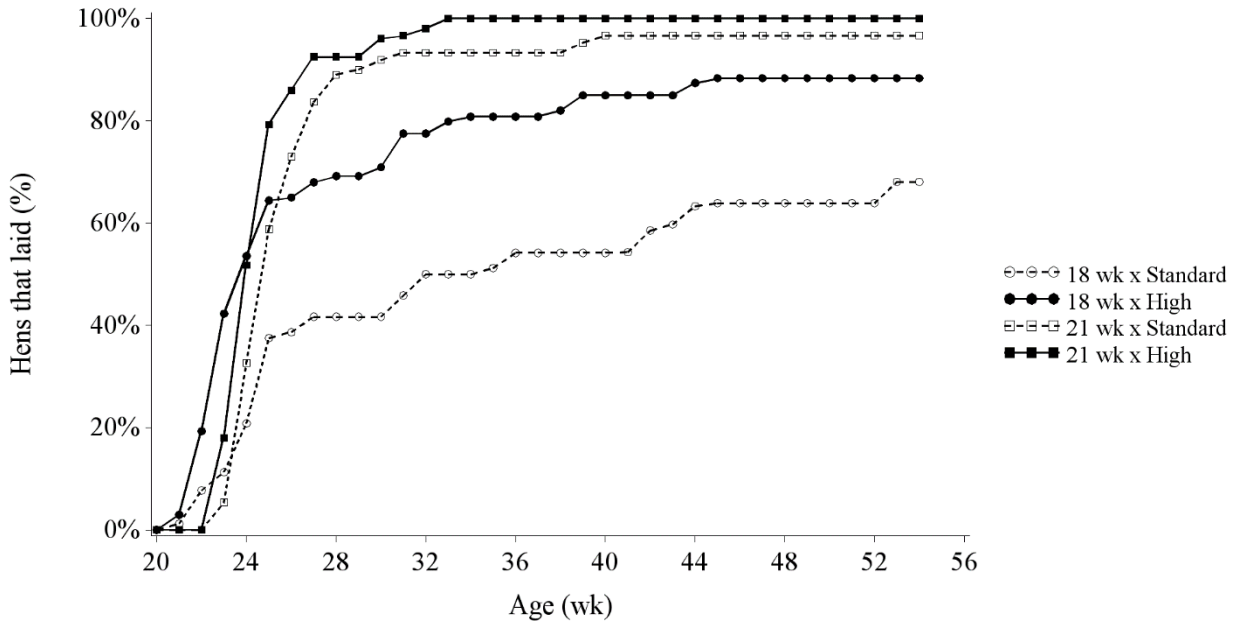

Figure 4.3 Percentage of hens that had laid their first egg fed towards either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and photostimulated at wk 18 or wk 21.

CHAPTER 5.

The effect of rearing photoperiod on broiler breeder reproductive performance depended on body weight[4]

5.1 Abstract

Body weight and rearing photoperiod are important factors affecting sexual maturation rate and reproductive performance in broiler breeders. The current experiment used a 2 x 3 factorial arrangement of treatments to study the interaction between BW and rearing photoperiod on reproductive performance in group housed broiler breeder hens, while minimizing variation in BW. Hens (n = 180) were fed with a precision feeding system to allocate feed individually to achieve the breeder-recommended target curve (Standard) or to a target curve that reached the 21 wk BW at 18 wk (High). Hens were on 8L:16D, 10L:14D, or 12L:12D photoschedules during rearing and were photostimulated at 21 wk with a 16L:8D photoschedule. Sexual maturity (defined as age at first egg) and individual egg production to 55 wk were recorded. At 55 wk, proportional weights of individual body components were determined by dissection. Differences were reported as significant at $P \leq 0.05$. A significant interaction between BW and rearing photoschedule affected age at sexual maturity and egg production. In the High BW treatment, age at sexual maturity did not differ between hens and the 8L:16D and 10L:14D photoschedules (173 vs. 172 d, respectively). In the Standard BW treatment, the 12L:12D rearing photoperiod delayed sexual maturity compared with the 8L:16D rearing photoperiod (266 vs. 180 d, respectively). All hens on the High BW treatment laid at least one egg before the end of the experiment. Conversely, 3.3%, 18.1%, and 37.6% of Standard BW hens on the 8L:16D, 10L:14D, and 12L:12D photoschedules, respectively, never commenced egg production. At the end of the experiment, proportional breast weight was higher and proportional fatpad weight was lower in Standard compared to High BW hens (25.8% vs. 27.5% and 2.4% vs. 1.5% of BW, respectively). We conclude that increased BW partially counters the effect of longer photoschedules on sexual maturity in broiler breeders and that dissipation of the photorefractory state depends on BW.

[4] Published in Poultry Science, volume 97, issue 9, pages 3286-3294

5.2 Introduction

In broiler breeders, rearing photoperiod and BW both affect sexual maturation and productivity, and to date those effects have been reported as independent. Photoperiod needs to be controlled during rearing to dissipate juvenile photorefractoriness. Under natural conditions, juvenile photorefractoriness occurs when pullets are exposed to long photoperiods (≥ 13 h), which prevents birds from becoming sexually mature in the same year in which they are hatched, thus avoiding offspring in suboptimal conditions (Lewis, 2006). In broiler breeders, rearing photoperiod determines the dissipation rate of the photorefractory state and the age at sexual maturation (Payne, 1975; Lewis et al., 2004). Exposure to short rearing photoperiods (≤ 10 h), accelerates dissipation of the photorefractory state and synchronizes onset of lay after photostimulation. However, under long rearing photoperiods (≥ 13 h), sexual maturity is delayed and egg production is reduced (Lewis et al., 2003; Lewis, 2006).

Independent of photoperiod, higher than recommended BW at the end of rearing accelerated sexual maturity (age at first egg), whereas a lower than recommended BW delayed sexual maturity (Fattori et al., 1991; Renema et al., 2001a,b; Hocking, 2004; Ekmay et al., 2012). However, other studies did not find the same result (Zuidhof et al., 2007; van Emous et al., 2013). Target BW curves of the latter studies converged at peak production, whereas target BW curves in the former were not aligned during the laying period (van Emous et al., 2013). Therefore, both target BW and the timing and level of feed restriction may affect sexual maturity and reproductive efficiency.

Earlier studies reported effects of rearing photoperiod as independent of BW (Gous and Cherry, 2004; Lewis et al., 2004, 2005). However, over the past decades, variation in BW in group housed flocks has increased as a result of increased levels of feed restriction and feed competition (Renema et al., 2007). Previous studies investigating the interaction between rearing photoperiod and BW were performed on hens reared in groups. In these studies, high within-treatment BW variation may have overshadowed interactions between BW and photoperiod treatments.

Therefore, the aim of the current research was to investigate the interaction between BW and rearing photoperiod on group housed broiler breeder reproductive performance with minimal BW variation. It was hypothesized that onset of lay would be delayed in lower BW hens under extended photoperiods, and egg production would be reduced. Conversely, within photoschedule

treatments, higher BW hens would dissipate photorefractoriness and mature more quickly, thereby increasing total egg production.

5.3 Materials and methods

5.1.1 Experimental design

The animal protocol for the study was approved by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care Guidelines and Policies (CCAC, 2009). The experiment was conducted as a 2 x 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 BW at 18 wk (High), and maintained under 8L:16D, 10L:14D, or 12L:12D photoschedules during rearing. The resulting High target BW was 22% higher than the Standard target BW at 21 wk of age.

5.3.1 Animals and housing

Ross 708 broiler breeder chicks (n=180; provided by Aviagen, Huntsville, Alabama, USA) were neck tagged for individual identification, and randomly allocated in 6 environmentally controlled rooms measuring 3.8 × 2.2 m (30 chicks per room). Floors of the rooms were covered with wood shavings at an approximate depth of 5 cm. Each room was equipped with a precision feeding (PF) system (Zuidhof et al., 2016, 2017), which controlled individual feed intake to achieve and adhere to the assigned target BW curves. Water was provided *ad libitum* with nipple drinkers during the entire experiment and a fountain style supplemental drinker was provided in each pen during the first wk. From d 0 to 16, birds were trained to use the PF system and were fed *ad libitum*. At d 16, birds were tagged with a radio frequency identification (RFID) wing band, 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 and were allowed access to feed for a duration of 45 s when their BW, measured in real-time by the PF system, was lower than their treatment target BW. When their measured BW was equal to or higher than their treatment target, birds were ejected from the PF system. 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 two 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 (2,900 AME, 19% CP, 1.1% Ca); from wk 4 to wk 23 pullets received a wheat and barley based grower diet (2,589 AME, 14.2% CP, and 0.9% Ca); from wk 23 to wk 34 hens received a wheat based peak layer diet (2,689 AME, 15.0% CP, and 3.3% Ca); and from wk 35 to wk 55 hens received a wheat based post peak layer diet (2,682 AME 14.6% CP, and 3.3% Ca).

At wk 18, a nest box with 8 nesting sites equipped with RFID readers was installed in each room, which identified eggs of individual hens. At wk 21, 3 roosters were introduced to each room. Roosters had been reared in a separate location under an 8L:16D photoschedule and precision fed on their breeder-recommended target BW curve (Aviagen, 2016).

5.3.2 Data collection

For the first two wk, pullets were weighed manually on a daily basis to confirm growth and use of the PF system. Birds that were not growing were trained individually to use the PF system. After individual feeding started, the PF system recorded individual BW and feed intake on a per visit basis. Feed intake and visit frequency was checked on a daily basis to ensure all birds were accessing the PF system. Because it would not be possible for floor eggs to be linked with individual hens because hens on different BW treatments were housed in the same room, cloacae of all hens were palpated daily to detect hard-shelled eggs in the shell gland to measure age at first egg and individual egg production from 20 wk to 36 wk. This ensured a precise estimate of age at first egg for each individual bird. As the majority of the birds on the 8L:16D photoschedule treatment had entered lay by wk 36, from 36 wk onward, daily palpation was performed every second wk. Eggs assigned to individual hens were weighed daily. Eggs between 40 g and 90 g were included in statistical analysis for egg weight. Eggs weighing more than 90 g were considered double yolked eggs and were analyzed separately. Mortality (including cull) was recorded throughout the experiment. At wk 55, all remaining hens were killed by cervical dislocation and dissected. Abdominal fat pad, filled gastro-intestinal tract (GIT), breast muscle (total weight of pectoralis major and pectoralis minor), heart, liver, oviduct (without content), and ovary weight were recorded. The GIT consisted of the complete digestive tract including pancreas, from 2 cm anterior to the crop up to but not including the bursa, with fat adhering to the proventriculus and

gizzard removed (included in abdominal fatpad weight). In addition, the number of large yellow follicles (LYF) was recorded.

5.3.3 Statistical analysis

All ANOVA were conducted using the MIXED procedure of SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012). Pairwise differences between means were determined with the PDIFF option of the LSMEANS statement and were considered significant at $P \leq 0.05$. Tukey's range test was used to compare treatment means. Hen was the experimental unit, except for cumulative hens in lay and percentage of hens that did not commence egg production before wk 55. For the latter, hens within each BW treatment within each chamber were randomly assigned to one of 3 groups, after which the parameters were calculated per group and group was used as experimental unit. The model used for the coefficient of variation for BW (BW CV), egg production, cumulative hens in lay, and egg weight data included BW treatment, rearing photoschedule, and age as fixed effects and all 2 and 3-way interactions. Random variation due to hen was accounted for in all serial measurements. As a result of insufficient data points early in lay, egg weight was analyzed from wk 30 onward. The model used for age at first egg, BW at age at first egg, percentage of hens that did not commence egg production before wk 55, cumulative egg production, and cumulative feed intake data included BW treatment and rearing photoschedule as fixed effects, and their interaction. The model used for the dissection data included BW treatment and rearing photoschedule as fixed effects, and their interaction and a binary random variable indicating whether the hen had laid her first egg.

5.4 Results and discussion

5.4.1 BW, BW variation, and feed intake

Actual Standard and High BW profiles closely matched their target profiles up to 24 wk of age (Figure 5.1). BW CV throughout the experiment (Table 5.1) was dependent on age ($P = 0.015$) and on BW treatment ($P = 0.003$). As no significant pair-wise differences were indicated after Tukey's range test, Table 5.1 shows results of the least significant difference test. BW CV of the High BW treatment increased after photostimulation compared to the Standard BW treatment. We hypothesize that this was mainly because hens on the High BW treatment started laying earlier compared to hens on the Standard BW treatment and sexually matured at a BW below their target BW (Table 5.3; Figure 5.1). These hens remained at a BW lower than their target throughout the study, suggesting that they reached their mature BW. As their mature BW was lower than the

target, BW variability increased in the High BW treatment. By using the PF system, the BW CV in our experiment was well below the 8% to 15% reported in recent studies on the effects of photoperiod or BW on broiler breeder performance (Gous and Cherry, 2004; van Emous et al., 2013; Zuidhof et al., 2015), and lower than reported BW CV in a previous study using a PF system (Zuidhof et al., 2017).

For cumulative feed intake (CFI), there was a significant interaction between BW and rearing photoschedule. During the rearing phase, CFI was lower for hens on the Standard BW treatment compared to hens on the High BW treatment (Table 5.2). This was anticipated as lower energy and protein requirements are expected for growth and maintenance for hens fed to a lower BW target. Within the High BW treatment, CFI was lower in hens on the 8L:16D photoschedule compared to hens on the 10L:14D and 12L:12D photoschedule. Gous and Cherry (2004) also showed an interaction between rearing photoperiod and BW target on CFI, but a clear explanation for the result was not evident. In their study, CFI was 110 and 770 g higher in the 8L:16D treatment compared to the 17L:7D treatment for the 1,550 g and 2,500 g 21 wk target, however, for the 2,150 g and 2,850 g 21 wk target, CFI was 160 g and 360 g lower in the shorter photoperiod treatment. This is not consistent with the current results, however, their long photoperiod was 5 hours longer than in the current study. We hypothesize that increased photoperiod beyond 8L:16D increased the period of activity of the pullets during the 24 h period, which might have increased the energetic expenditure for locomotion. We recognize that further investigation into energy expenditure and energy allocation is needed. During the laying phase, CFI of hens on the High BW target did not differ, whereas CFI of hens on the Standard BW target was reduced in the 10L:14D and 12L:12D rearing photoschedules compared to the 8L:16D rearing photoschedule. This latter interaction was likely the result of differences in egg production, and respective increase in ME intake to support egg production (Romero et al., 2009). Mortality throughout the trial did not differ significantly between treatment groups.

5.4.2 Sexual maturity and egg production

In line with previous findings, increased BW accelerated sexual maturity (Table 5.3). Hens on the High BW treatment started laying 34 d earlier than hens on the Standard BW treatment. The advance of sexual maturity was 1.6 d, 9.1 d, and 12.7 d per 100 g increase in BW at 20 wk of age for the 8L:16D, 10L:14D, and 12L:12D rearing photoschedule, respectively. For the 8L:16D rearing photoschedule, similar observations were previously reported, where sexual maturity

advanced between 1.5 d and 3.0 d per 100 g increase in BW at 20 wk of age (Renema et al., 2001a; Gous and Cherry, 2004; Sun and Coon, 2005). Conversely, Fattori et al. (1991) showed that a decrease in BW of 100 g at 20 wk delayed sexual maturity by 7.3 d. The latter suggests that there is a minimum BW threshold that needs to be met for sexual maturation to proceed. However, this threshold might depend upon rearing photoperiod, as there was an interaction between rearing photoperiod and BW treatment on BW at sexual maturity (Table 5.3).

Sexual maturity was delayed and egg production reduced in hens reared on increased photoperiods (Table 5.3; Figure 5.2), similar to the results of Lewis et al. (2003). Hens reared on 10L:14D tended to start laying later than hens on the 8L:16D photoschedule (15 d, $P = 0.082$) and hens reared on the 12L:12D photoschedule started laying 61 d later ($P < 0.001$) than hens on the 8L:16D photoschedule. This confirms that modern broiler breeders are photorefractory at hatch, and that the photorefractory state was dissipated by a short photoperiod in a photoperiod dependent manner.

In contrast with Lewis (2006), we found that the effect of rearing photoschedule on sexual maturity and egg production was dependent on BW (Table 5.3; Figure 5.2). High BW x 8L:16D or 10L:14D hens did not differ in age at sexual maturity (173 vs. 172 d, respectively), but Standard BW hens showed a significant delay in sexual maturity when photoperiod increased from 8L:16D to 12L:12D (180 vs. 266 d, respectively), while the 10L:14D treatment was intermediate (212 d). In addition, Figure 5.2 shows that the difference in egg production within rearing photoschedule was greater for Standard BW hens compared with High BW hens. For the subset of hens that were laying, productivity did not differ between treatments (data not shown), thus, the difference in egg production originated from the rate (%) of hens reaching sexual maturity within each group (Figure 5.3). As a matter of fact, by the end of the experiment all High BW hens started laying, whereas within the Standard BW treatment 3.3%, 18.1%, and 37.6% of hens did not commence egg production under the 8L:16D, 10L:14D, and 12L:12D photoschedule, respectively (Table 5.3). In contrast with previous literature, the current study took a vastly different approach in the method of feeding used to control BW variation. First, the increased precision in which we were able to control BW and thus reduce BW CV with the PF system may have resulted in the ability to show the interaction between BW and rearing photoperiod. Second, the PF system provided an increased frequency of meals time-separated over the day and reduced meal size compared to conventional feeding methods. Feed allocation strategy also differed during the period where hens were

expected to sexually mature, as there was no production-related feed increase. Feed allocations were provided to achieve the treatment-specific BW targets. Therefore, an individual hen would receive an additional feed allowance to support egg production only when her first egg was laid because in real-time, BW was reduced by the act of oviposition. We hypothesize that the combination of these factors might have altered the metabolism of the hens, potentially restricting hens to the point where metabolic triggers to sexually mature were absent or remaining suppressed, causing the interaction. This suggests that current breeder recommended BW targets may not allow for sufficient body reserves required for the onset of lay, at least in the precision feeding scenario implemented in the current study.

Our results suggest that a stronger metabolic signal resulting from a higher positive energy balance in the High BW treatment countered negative signals caused by extended rearing photoperiods. A lower metabolic signal in the Standard BW treatment reduced reproductive axis responsiveness, and delayed sexual maturation. This hypothesis is consistent with the commercial practice of 'challenge feeding'. The industry has observed for some time that increased feed intake allowance around the time egg production increases to 60 to 70% can stimulate egg production in the short term, presumably by bringing more hens into lay (Coon, 2002). The potential problem with challenge feeding is that it may reduce the persistency of lay for those hens already laying. Therefore, we suggest that it would be more advantageous to remove as many inhibitory reproductive signals as possible, instead of applying challenge feeding to synchronize sexual maturation.

As the integration center for the control of the reproductive axis is located within the hypothalamus, it has been suggested that the hypothalamus is a logical target for metabolic reproductive signals (Bédécarrats et al., 2016). Unpublished results from our laboratory indicate that hens with increased ME intake enter lay earlier with higher gonadotropin releasing hormone and lower gonadotropin inhibitory hormone expression in the hypothalamus compared to hens with a below average ME intake. Therefore, we hypothesize that whether direct or indirect, metabolic cues can alter the balance between stimulatory and inhibitory output from the hypothalamus to the pituitary gland. However, further studies are required to identify the pathways and mechanisms involved.

5.4.3 Egg weight

Egg weight increased with age for all treatments ($P < 0.001$), independent of BW treatment or rearing photoschedule (data not shown). Number of eggs less than 40 g and number of double yolked eggs did not differ between treatments (data not shown). There was a significant interaction between the effect of BW and rearing photoschedule on egg weight ($P < 0.001$). Egg weight was not different between the 8L:16D and 12L:12D photoschedules, but for the 10L:14D photoschedule, egg weight of Standard BW hens was 1.5 g lower compared with High BW hens (Table 5.3). No effects of rearing photoperiod on egg weight have been reported before. Previous studies showed that an increased 20 wk BW target did not affect egg weight (Fattori et al., 1991; Hocking et al., 2001, 2002; Gous and Cherry, 2004; Robinson et al., 2007; Ekmay et al., 2012; van Emous et al., 2013), where others showed that a 20% increased BW at 20 wk of age increased egg weight by about 1 g (Renema et al., 2001a; b; Sun and Coon, 2005). We are currently unsure about the possible explanation of the described interaction.

5.4.4 Body conformation

Proportional weight of body parts and number of LYF at 55 wk are reported in Table 5.4. Standard BW hens had a higher proportional breast weight and a lower proportional fatpad weight compared with High BW hens (27.5% vs. 25.8%; $P = 0.006$, and 1.5% vs. 2.4%; $P < 0.001$), respectively), which coincided with lower egg production. In addition, Standard BW hens had a lower proportional ovary weight and lower number of LYF compared to High BW hens. We hypothesize that lower egg production and delayed onset of lay in the Standard BW hens compared with High BW hens may have originated from an insufficient proportion or absolute amount of lean or fat mass. Although body part weights were not analyzed at photostimulation, we hypothesize that greater body mass accretion in High BW hens enhanced their reproductive readiness at the time of photostimulation, and these hens reached their minimum lean or fat mass thresholds earlier than Standard BW hens. Body composition, either (proportional) lean mass or fat mass, has been proposed as a factor partially responsible for age at sexual maturation and egg production in both laying hens and broiler breeders (Kwakkel et al., 1991, 1995; Lesuisse et al., 2017). Hens that did not commence egg production before wk 55 had a 3.7% (of BW) greater proportion of breast and a 0.9% (of BW) smaller proportion of fatpad (Table 5.4). Thus, hens not in lay had 1.15 times more breast muscle and 0.63 of the abdominal fatpad of hens that had laid eggs. This suggests a

deficiency of fat rather than a lean mass threshold. Therefore, a fat threshold mass required for the onset of lay may not have been achieved by almost one fifth of the Standard BW hens in our study. Hens on the High BW treatment had similar egg production to the breeder performance objectives (Aviagen, 2016), which indicates that the Standard target BW curve was actually sub-optimal, at least for precision-fed broiler breeders. High BW hens that weighed consistently less than the target BW were fed every time they entered the feeding station during the laying phase. Since these hens matured at a BW below the High target BW curve, their feed intake was unrestricted during lay. After meeting their maintenance ME requirements, their egg production potential would not have been limited by restricted ME intake, in contrast to what would have been the case for Standard BW hens.

The discrepancy between the performance of the hens on the Standard BW treatment and the breeder performance objectives (Aviagen, 2016) could have originated from differing body composition due to the feeding method. Zuidhof et al. (2015) showed that an increase in feeding frequency from skip-a-day to daily feeding increased breast muscle and reduced abdominal fat pad weights in breeder pullets. Compared to conventional feeding methods, feeding frequency in the current experiment was high, as the PF system fed individual hens small meals multiple times per day. Zuidhof et al. (2017) explained that high feeding frequency makes nutrients available from the gut throughout the day. This might reduce the metabolic incentive for hens to store energy in the form of fat and instead stimulate growth of lean body tissue. The energy stores in fat are required for egg production as production of yolk-lipids and albumen protein requires energy. Therefore, insufficient fat stores in Standard BW hens could have delayed onset of lay. Consistent with this hypothesis, Lewis et al. (2003) compared hens from male and female breeder lines and showed that the leaner male line had a delayed sexual maturity compared to female line, independent of rearing photoschedule (223 vs. 207 d, respectively). In addition, in human medicine, there is a growing body of literature that describes that an early onset of sexual maturity coincides with an increased body fat percentage in women (Walvoord, 2010).

5.5 Conclusions

To our knowledge, this is the first time an interaction has been shown between the effects of BW and rearing photoperiod on reproductive performance in broiler breeders. These results suggest that greater BW or feed intake might override negative signals such as increased photoperiods against sexual maturation. In addition, increasing the target BW for breeder hens could increase

egg production, particularly when managing BW with a precision feeding system, and might counteract the negative effects of increased photoperiod during rearing in open housed facilities. We suggest further studies to investigate body fat thresholds in broiler breeders. In addition, we suggest further investigation into physiological and neuroendocrinological cues behind the effects of BW and rearing photoperiod.

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5.8 Tables

Table 5.1 Coefficient of variation for BW (BW CV) at various ages of hens fed with a precision feeding system to achieve a High or Standard BW[1] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS).

			BW CV (%)													
	BW	RPS	Wk 4	SEM	Wk 7	SEM	Wk 14	SEM	Wk 21	SEM	Wk 27	SEM	Wk 40	SEM	Wk 54	SEM
Wk[2]			3.6^{w}	0.6	2.5^{wx}	0.6	0.7^{z}	0.6	0.8^{yz}	0.6	1.7^{xyz}	0.6	2.4^{wxy}	0.6	2.7^{wx}	0.6
BW x wk	High		4.3	0.7	2.3	0.7	0.9	0.7	0.5	0.7	2.7^{a}	0.7	3.6^{a}	0.7	3.4	0.7
	Standard		2.8	0.7	2.8	0.7	0.5	0.7	1.1	0.7	0.8^{b}	0.7	1.2^{b}	0.7	2.0	0.7
RPS x wk		8L:16D	4.2	1.0	2.7	1.0	0.3	1.0	1.0	1.0	2.7	1.0	4.0	1.0	3.3	1.0
		10L:14D	1.9	1.0	2.9	1.0	0.4	1.0	0.3	1.0	1.6	1.0	2.2^{a}	1.0	3.7	1.0
		12L:12D	4.6	1.0	2.0	1.0	1.3	1.0	1.1	1.0	0.9	1.0	1.0	1.0	1.0	1.0
BW x RPS x wk	High	8L:16D	4.9^{ab}	1.2	2.5	1.2	0.2	1.2	0.8	1.2	4.1^{a}	1.2	5.4^{a}	1.2	4.6^{a}	1.2
		10L:14D	2.2^{b}	1.2	3.1	1.2	0.4	1.2	0.3	1.2	2.6^{ab}	1.2	4.0^{ab}	1.2	4.0^{a}	1.2
		12L:12D	5.9^{a}	1.2	1.3	1.2	2.2	1.2	0.3	1.2	1.3^{ab}	1.2	1.4^{bc}	1.2	1.4^{ab}	1.2
	Standard	8L:16D	3.6^{ab}	1.2	2.8	1.2	0.4	1.2	1.2	1.2	1.2^{b}	1.2	2.6^{bc}	1.2	2.0^{ab}	1.2
		10L:14D	1.5^{b}	1.2	2.7	1.2	0.5	1.2	0.3	1.2	0.6^{b}	1.2	0.5^{c}	1.2	3.4^{ab}	1.2
		12L:12D	3.3^{ab}	1.2	2.8	1.2	0.5	1.2	1.8	1.2	0.5^{b}	1.2	0.7^{bc}	1.2	0.6^{b}	1.2

$^{a-c}$ LSMeans within a column and treatment group lacking a common superscript differ ($P \leq 0.05$).
$^{w-z}$ LSMeans within a row and treatment group lacking a common superscript differ ($P \leq 0.05$).
[1] Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).
[2] P - values for the different sources of variation were as follows: Wk, P = 0.015; BW, P = 0.003; RPS, P = 0.214; BW x RPS, P = 0.584; Wk x BW, P = 0.056; Wk x RPS P = 0.377; Wk x BW x RPS, P = 0.714.

Table 5.2 Cumulative feed intake (CFI) of broiler breeder hens from d 16 to wk 21 (Rearing phase) and from wk 21 to wk 55 (Laying phase), fed to achieve a High or Standard BW[1] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS)

			Rearing phase		Laying phase	
	BW	RPS	CFI (g)	SEM	CFI (g)	SEM
BW	High		9,063[a]	36	33,718[a]	572
	Standard		7,337[b]	35	26,286[b]	561
RPS		8L:16D	8,091[b]	41	32,324[a]	651
		10L:14D	8,260[a]	46	30,505[a]	731
		12L:12D	8,249[a]	44	27,177[b]	698
BW x RPS	High	8L:16D	8,865[b]	58	34,652[a]	920
		10L:14D	9,157[a]	67	34,939[a]	1,069
		12L:12D	9,166[a]	61	31,563[ab]	976
	Standard	8L:16D	7,316[c]	58	29,996[b]	920
		10L:14D	7,364[c]	62	26,072[c]	997
		12L:12D	7,331[c]	62	22,791[c]	997
Source of variation			P value		P value	
BW			< 0.001		< 0.001	
RPS			0.008		< 0.001	
BW x RPS			0.008		0.044	

[a-c] LSMeans within a column and treatment group lacking a common superscript differ ($P \leq 0.05$).

[1] Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

Table 5.3 Age at first egg (AFE)[1], percentage of hens that did not commence production before 55 wk (Not laid), BW at AFE[1], cumulative egg production (Eggs), and overall egg weight of hens fed to achieve a High or Standard BW[2] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS).

	BW	RPS	AFE (d)	SEM	Not laid (%)	SEM	BW at AFE (g)	SEM	Eggs	SEM	Egg weight (g)	SEM
BW	High		185^{b}	5	0.0^{b}	2.07	3,278^{a}	33	138^{a}	6	64.8	0.1
	Standard		219^{a}	5	19.7^{a}	2.07	3,057^{b}	37	88^{b}	6	64.4	0.2
RPS		8L:16D	177^{b}	5	1.7^{b}	2.53	2,935^{b}	38	138^{a}	7	65.2^{a}	0.1
		10L:14D	192^{b}	6	9.0^{b}	2.53	3,053^{b}	45	120^{a}	8	63.4^{b}	0.1
		12L:12D	238^{a}	6	18.8^{a}	2.53	3,513^{a}	45	81^{b}	7	65.1^{a}	0.1
BW x RPS	High	8L:16D	173^{c}	8	0.0^{c}	3.58	3,137^{b}	53	152	10	64.8^{ab}	0.2
		10L:14D	172^{c}	9	0.0^{c}	3.58	3,074^{b}	63	151	12	64.2^{b}	0.3
		12L:12D	210^{b}	8	0.0^{c}	3.58	3,621^{a}	55	111	10	65.3^{a}	0.2
	Standard	8L:16D	180^{bc}	8	3.3^{bc}	3.58	2,732^{c}	54	125	10	65.6^{a}	0.2
		10L:14D	212^{b}	9	18.1^{b}	3.58	3,033^{b}	65	89	11	62.7^{c}	0.3
		12L:12D	266^{a}	10	37.6^{a}	3.58	3,405^{a}	71	51	10	64.9^{ab}	0.3
Source of variation			P – value									
BW			< 0.001		< 0.001		< 0.001		< 0.001		0.087	
RPS			< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
BW x RPS			0.012		< 0.001		0.010		0.160		< 0.001	

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

Table 5.4 Breast, fatpad, liver, heart, gastro intestinal tract (GIT), ovary and oviduct weight as percentage of live BW, and number of large yellow follicles (LYF) of hens at 55 wk fed to achieve a High or Standard BW[1] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS) and that either commenced egg production (Laid) or did not commence egg production (Not laid) before wk 55[2].

	BW	RPS	Breast (%)	SEM	Fatpad (%)	SEM	Liver (%)	SEM	Heart (%)	SEM	GIT (%)	SEM	Ovary (%)	SEM	Oviduct (%)	SEM	LYF	SEM
BW	High		25.8^{b}	0.64	2.4^{a}	0.26	1.7^{a}	0.09	0.36	0.017	4.8	0.15	1.6^{a}	0.22	0.9	0.12	4.4^{a}	0.47
	Standard		27.5^{a}	0.48	1.5^{b}	0.19	1.6^{b}	0.07	0.34	0.013	4.9	0.11	0.9^{b}	0.17	0.9	0.09	2.9^{b}	0.37
RPS		8L:16D	26.1	0.68	2.1	0.27	1.9^{a}	0.09	0.38^{a}	0.018	5.0	0.15	1.3	0.24	0.9	0.13	3.8	0.49
		10L:14D	27.2	0.62	1.7	0.25	1.7^{b}	0.08	0.35^{ab}	0.016	4.8	0.14	1.1	0.22	0.9	0.11	3.9	0.46
		12L:12D	26.6	0.58	2.1	0.23	1.4^{c}	0.08	0.32^{b}	0.015	4.7	0.13	1.3	0.20	0.9	0.11	3.3	0.44
BW x RPS	High	8L:16D	25.6	0.85	2.5	0.34	2.0	0.12	0.39	0.022	5.0	0.19	1.6	0.30	0.9	0.16	4.7	0.61
		10L:14D	26.6	0.85	2.2	0.34	1.7	0.12	0.37	0.022	4.7	0.19	1.4	0.30	0.9	0.16	4.8	0.61
		12L:12D	25.3	0.85	2.7	0.34	1.4	0.12	0.32	0.022	4.7	0.19	1.8	0.30	0.9	0.16	3.8	0.61
	Standard	8L:16D	26.6	0.82	1.6	0.33	1.8	0.11	0.38	0.021	5.1	0.19	1.1	0.29	0.8	0.15	3.0	0.57
		10L:14D	27.9	0.73	1.3	0.29	1.6	0.10	0.34	0.019	5.0	0.17	0.7	0.26	0.9	0.14	3.1	0.55
		12L:12D	28.0	0.70	1.5	0.28	1.3	0.10	0.32	0.018	4.6	0.16	0.8	0.25	1.0	0.13	2.7	0.53
Laid			24.8^{b}	0.30	2.4^{a}	0.12	1.8^{a}	0.04	0.36	0.008	4.7	0.07	1.8^{a}	0.11	1.6^{a}	0.06	5.6^{a}	0.21
Not laid			28.5^{a}	0.93	1.5^{b}	0.37	1.5^{b}	0.13	0.34	0.024	5.0	0.21	0.7^{b}	0.32	0.2^{b}	0.17	1.8^{b}	0.72
Source of variation			P – value															
BW			0.006		< 0.001		0.044		0.379		0.587		0.001		0.912		0.001	
RPS			0.253		0.347		< 0.001		0.002		0.064		0.556		0.930		0.323	
BW x RPS			0.423		0.703		0.768		0.608		0.396		0.633		0.717		0.774	
Laid			< 0.001		0.036		0.005		0.347		0.274		0.004		< 0.001		< 0.001	

[a-c] LSMeans within a column and treatment group lacking a common superscript differ ($P \leq 0.05$).

[1] Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

[2] The effect of whether hens had commenced egg production or not before wk 55 on body composition did not depend on rearing photoschedule.

5.9 Figures

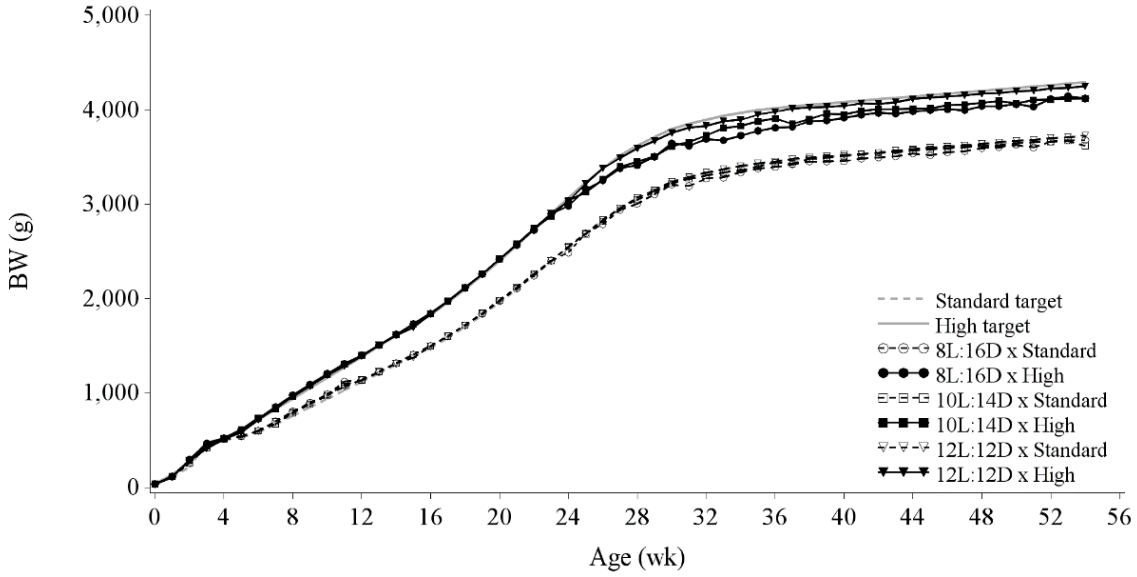

Figure 5.1 BW of hens fed to achieve either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule.

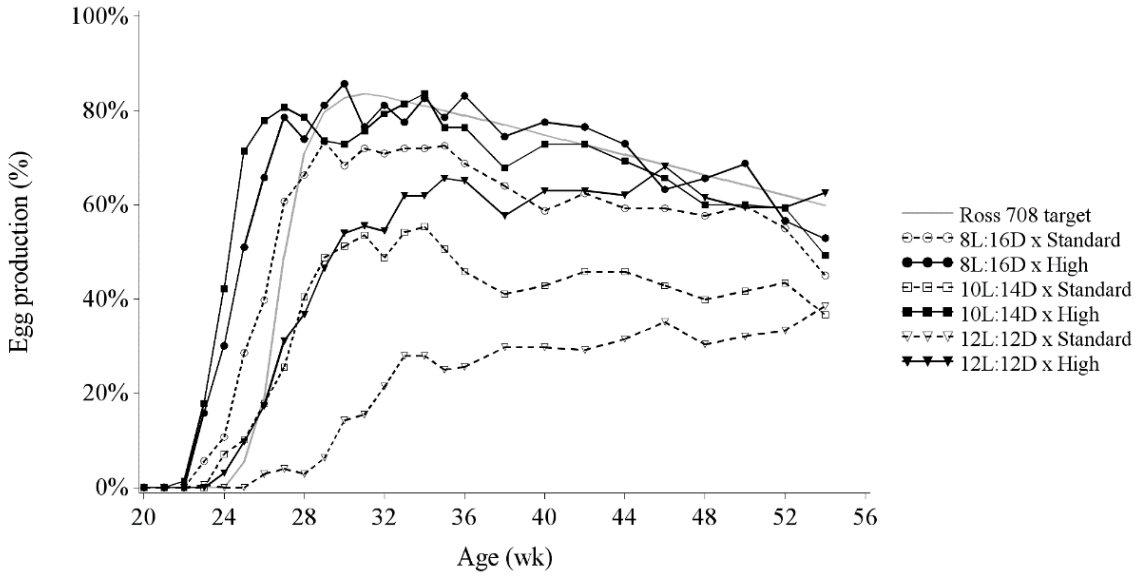

Figure 5.2 Hen day egg production of hens fed to achieve either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule.

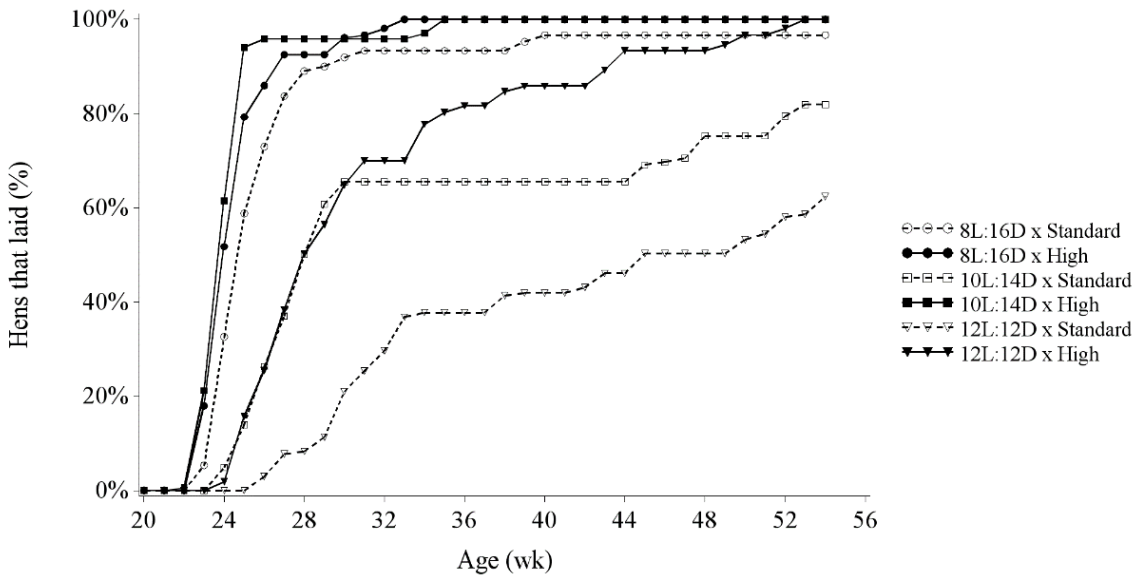

Figure 5.3 Percentage of hens that had laid their first egg. Hens were fed to achieve either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D rearing photoschedule.

CHAPTER 6.

A model of pre-pubertal broiler breeder estradiol-17β levels predicts advanced sexual maturation for birds with high body weight or short juvenile daylength exposure[5]

6.1 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 x 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 wk 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 illustrate an example for other studies in endocrinological research in poultry reproduction.

[5] Published in Poultry Science volume 98, issue 10, pages 5137-5145

6.2 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 has 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; Chapter5). 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; Chapter 5). 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.

6.3 Materials and methods

6.3.1 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 x 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.

6.3.2 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 24h/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 two 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 (2,900 AME, 19% CP, 1.1% Ca); from wk 4 to wk 23 pullets received a wheat and barley based grower diet (2,589 AME, 14.2% CP, and 0.9% Ca); from wk 23 to wk 34 hens received a wheat based peak layer diet (2,689 AME, 15.0% CP, and 3.3% Ca).

6.3.3 Data collection

A detailed description of data collection methods can be found in van der Klein et al. (2018; Chapter 5). 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 photoschedule treatment had entered lay by wk 36 thus, from 36 wk onward, daily palpation was performed every second wk.

6.3.4 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 x 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 4ºC 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 20ºC 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 -20ºC until assay. Plasma E2 was measured in thawed extracted plasma samples using the DetectX 17ß-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.

6.3.5 Design of models

Two mixed nonlinear models were considered using either the complete E2 dataset or a subset of all E2 data. The subset of E2 data only contained hens for which AFE was within 100 d of photostimulation and is referred to as photosensitive hens. The reason for distinguishing hens based on photosensitivity was that visual analysis of the data (Figure 6.1) 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; Chapter 5). Both models described E2 levels as a function of age in wk and were based on a Gompertz growth curve, including the natural logarithm (e; 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. The E2 inflection point was used as reference parameter as it was directly obtainable as the individual estimates from the fitted models (t_{inf} as mathematical and biologically relevant parameter).

6.3.6 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. Nonlinear 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:

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

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

6.4 Results and discussion

6.4.1 Animal performance

Detailed description of animal performance such as feed intake, BW, and AFE was reported previously in van der Klein et al. (2018; Chapter 5). 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).

6.4.2 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] fitted the data better, as BIC and AIC values were 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 6.2 shows a visualization of the fitted model parameters from Table 6.1. 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.

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.

6.4.3 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 6.1). 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 6.2), 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 (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 more 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 wk 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 weeks 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 24h 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, 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 6.2). 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.

6.5 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 predicted 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 predicted 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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6.8 Tables

Table 6.1 Functional specifications, coefficients, and fit statistics criteria of the modified Gompertz models describing estradiol-17β (E2) levels as a function of age.

Equation	Chronological age model [1] $E_{it} = E_b + (E_m - E_b) * e^{-e^{-b\,(t-(t_{inf}+u_i))}} + \varepsilon_i$						Physiological age model [2] $E_{it} = E_b + (E_m - E_b) * e^{-e^{-b\,(t-AFE_i-(t_{inf}+u_i))}} + \varepsilon_i$					
	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

Table 6.2 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[ab]	0.051
BW x RPS	High	8L:16D	1.07[bc]	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[bc]	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[bc]	0.425	1.78[bc]	0.551	2.29	0.059
		10L:14D	1.44[b]	0.343	3.42[bc]	0.521	2.40[bc]	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 x 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).

6.9 Figures

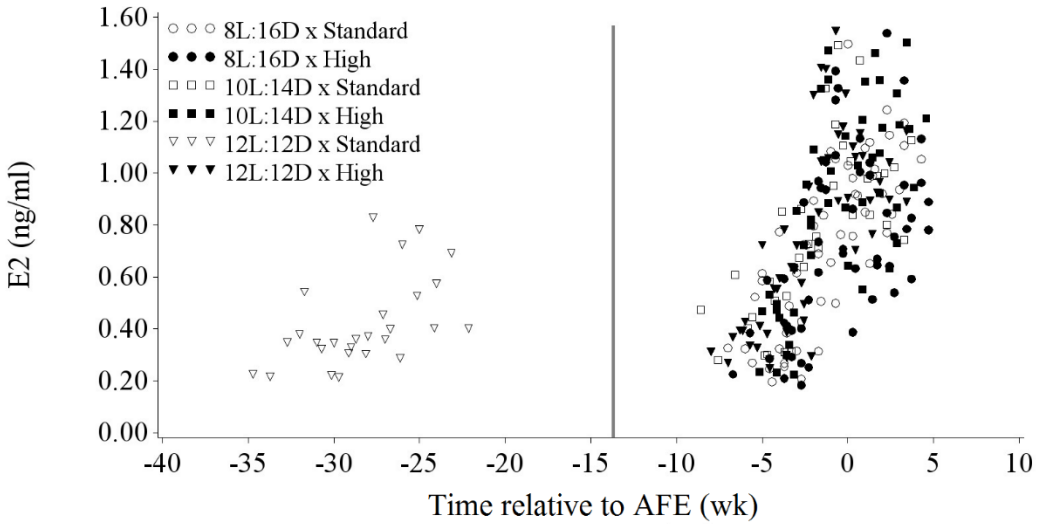

Figure 6.1 Estradiol-17β (E2) levels in broiler breeder hens relative to individual age at first egg (AFE, time=0 wk) between wk 20 and 28 and fed to achieve either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule. Individuals to the left of the grey vertical reference line at 14.28 wk before AFE were considered photorefractory, individuals on the right of the grey vertical line were considered photosensitive.

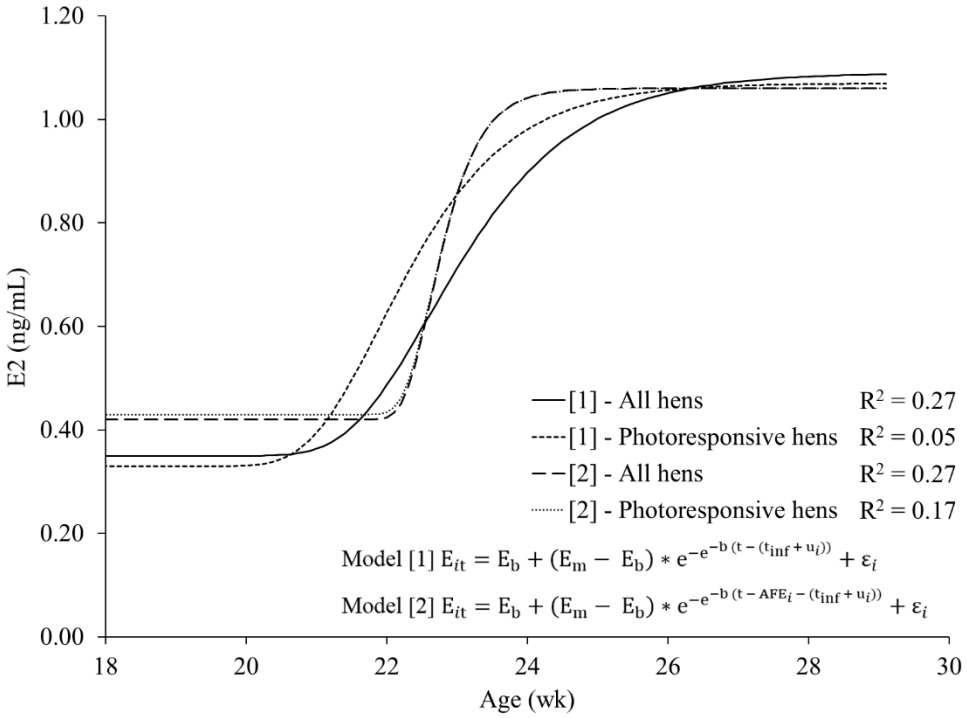

Figure 6.2 Comparison between predicted estradiol-17β (E2) levels in broiler breeder hens, modelled by a modified Gompertz curve including chronological age (Model [1]) or physiological age (age relative to age at first egg, Model [2], the average age at first egg of 25 wk was used), including all hens 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.

CHAPTER 7.

Modelling life-time energy partitioning in broiler breeders with differing body weight and rearing photoperiods[6]

7.1 Abstract

Understanding energy partitioning in broiler breeders is needed to provide efficiency indicators for breeding purposes. This study compared 4 nonlinear models partitioning ME intake to BW, average daily gain (ADG), and egg mass (EM) and described the effect of BW and rearing photoperiod on energy partitioning. Ross 708 broiler breeders (n = 180) were kept in 6 pens, controlling individual BW of free run birds with precision feeding stations. Half of the birds in each chamber were assigned to the breeder-recommended target BW curve (Standard) or to an accelerated target BW curve reaching the 21-wk BW at wk 18 (High). Pairs of chambers were randomly assigned to 8L:16D, 10L:14D, or 12L:12D rearing photoschedules and photostimulated with 16L:8D at wk 21. Model [I] was: $MEI_d = a \times BW^b + c \times ADG \times BW^d + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW in kg; ADG in g/d; EM in g/d. Models [II-IV] were nonlinear mixed versions of model [I], and included individual [II], age-related [III], or both individual and age-related [IV] random terms to explain these sources of variation in maintenance requirement (a). Differences were reported as significant at $P \leq 0.05$. The mean square error was 2,111, 1,532, 1,668, and 46 for models [I-IV] respectively, inferring extra random variation was explained by incorporating 1 or 2 random terms. Estimated ME partitioned to maintenance [IV] was 130.6 ± 1.15 kcal/$kg^{0.58}$, and the ME requirement for ADG and EM were 0.63 ± 0.03kcal/g/$kg^{0.54}$ and 2.42 ± 0.04 kcal/g, respectively. During the laying period, maintenance estimates were 124.2 and 137.4 kcal/$kg^{0.58}$ for Standard and High BW treatment, and 130.7, 132.2, 129.5 kcal/$kg^{0.58}$ for the 8L:16D, 10L:14D, or 12L:12D treatments, respectively. Although hens on the Standard BW treatment with a 12L:12D rearing photoschedule were most energetically conservative, their reproductive performance was the poorest. Model IV provided a new biologically sound method

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for estimation of life-time energy partitioning in broiler breeders including an age-related random term.

7.2 Introduction

Understanding of energy partitioning towards maintenance, growth, and egg production in broiler breeders is needed to develop optimal feeding programs and provide energy efficiency indicators for breeding purposes. Indirect calorimetry and the comparative slaughter technique have been often used to study energy partitioning in poultry (Birkett and de Lange, 2001). However, mathematical models have become increasingly popular to help understand energy partitioning. These energy partitioning models have focused previously on either the growing period (Sakomura et al., 2003; Pishnamazi et al., 2015; Hadinia et al., 2018) or the laying period (Pishnamazi et al., 2008; Romero et al., 2009b; Darmani Kuhi et al., 2011, 2019). Although energy partitioning estimates for each period separately are important, models could be improved including both phases such that the effect of age on energy partitioning and the efficiency of growth and egg production over their life-time can be studied. This would benefit nutritionist and breeding companies as understanding of energy partitioning would provide tools to minimize energy loss to heat. The metabolizable energy (ME) intake lost as heat or total heat production (HP), is equivalent to the ME for maintenance (ME_m; Zuidhof, 2019). ME_m requirements reported in the literature have been confounded by 1) individual variation and 2) different degrees of (age related) feed restriction during rearing and laying phase.

Several indicators have been used to determine the efficiency of growth and egg production in poultry, such as the feed conversion ratio (FCR), residual feed intake (RFI), or residual heat production (RHP), also known as residual maintenance ME requirements (RME_m; Willems et al., 2013). FCR is defined as the amount of feed consumed per unit of weight gain (FCR_g) or unit of egg production (FCR_{egg}; Skinner-Noble and Teeter, 2003). FCR does not account for the variability ME_m requirements, therefore, FCR_g increases with age and BW due to higher ME_m requirements. RFI is defined as the difference between actual and expected feed intake as predicted from the requirements for ME_m, BW gain, and egg production (Byerly, 1941; Luiting, 1990). Although RFI accounts for ME_m requirements, it does not account for the heat increment of feeding (Swennen et al., 2007). Therefore, RFI can penalize high producing animals, as they increase feed intake to support production, yet consequentially also have an increased heat increment of feeding. In breeding programs, selection for FCR or RFI may not necessarily focus on birds with increased

efficiency, but on birds with a faster growth rate or reduced feed intake (Willems et al., 2013). In addition, environmental factors affecting ME_m requirements may also bias estimates of FCR and RFI. RHP or RME_m is the residual of the linear relationship between ME_m requirement, also referred to as total HP, and ME intake (Romero et al., 2009a; Hadinia et al., 2018). RHP removes the confounding effect of ME intake, or feed intake, including the heat increment of feeding, and may therefore be a better indicator of biological efficiency in poultry (Romero et al., 2009a).

The above described efficiency indicators are both phenotypically and genetically correlated and the strength of this correlation depends on the age of the bird (Willems et al., 2013) and potentially environmental factors affecting ME_m requirements, such as temperature. For example, genetic correlations between FCR and RFI were lower in broilers assessed from 28 to 35 d (0.31) compared to broilers assessed from 35 to 42 d (0.84, Aggrey et al., 2010). In addition, the level and composition of gain changes during the life-time (Vignale et al., 2017; Salas et al., 2019), therefore ME_m requirements might change as a result of body composition changes (Sakomura et al., 2003). Pishnamazi et al. (2008) concluded that estimated ME_m requirements for broiler breeders reduced from wk 4 (~200 kcal/d) to wk 16 (~100 kcal/d), which was related to feed intake per unit of metabolic BW ($R^2 = 0.99$, $P < 0.001$). However, to our knowledge, models including an effect of age on ME_m have not been reported.

Energy partitioning is affected by dietary and environmental factors, such as lighting and feed allowance. In broilers, a substantial body of research has aimed to optimize lighting programs for growth to improve production efficiency (Arowolo et al., 2019). Increased scotoperiods (> 4h darkness) have been used as a method to reduce feed intake and stimulate compensatory growth, resulting in improved feed efficiency (Schwean-Lardner et al., 2012) and prevention of (metabolic) health issues (Schwean-Lardner et al., 2013). In contrast, lighting programs in broiler breeders aim almost exclusively at dissipating the photorefractory state to prepare pullets for photostimulation, sexual maturation, and egg production (Lewis, 2006). Lewis (2006) concluded that BW at wk 20 decreased by about 15 g for each hour of photoperiod increase for pullets provided the same feed allocation during rearing. It was hypothesized to be an effect of increased ME_m requirements with longer photoperiod, as broiler breeders reduced HP during the scotoperiod (Macleod et al., 1980). Broiler breeder pullets are kept under severe feed restriction to control BW and body composition and optimize reproductive performance. Lewis et al. (2005) found at the same level of production, birds allowed accelerated growth from wk 10 to achieve 2.1 kg at wk 17 had a higher FCR_{egg}

compared to birds reared to achieve 2.1 kg at wk 21. The birds achieving the 2.1 kg target at wk 17 had a higher BW at sexual maturity compared to birds that achieved the 2.1 kg at wk 21 (3.6 kg vs 3.4 kg), hence, their ME_m requirements would have been higher as well.

Data used to develop energy partitioning models and test energy efficiency have often been collected from caged birds to measure individual feed intake and individual egg production. However, under practical circumstances broiler breeders are housed free run in groups to facilitate natural mating. Novel technologies like the precision feeding (PF) system (Zuidhof et al., 2016, 2017) allow for the first time collection of feed intake and BW data from individual free run birds. The aim of this study was to compare 4 different models partitioning ME intake to BW, ADG, and EM over the life-time of group housed broiler breeders fed with the PF system. Model fit and bias were compared. The best fitting model was used to estimate RFI and RHP. It was hypothesized that FCR_g, FCR_{egg}, RFI, and RHP would be decreased in treatments with reduced photoperiod and reduced BW.

7.3 Materials and methods

7.3.1 Experimental design

The animal protocol for the study was approved by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care Guidelines and Policies (CCAC, 2009). The experiment was conducted as randomized block design of a 2 x 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 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 and the 565 g BW difference was maintained from d 193 to the end of the study. Rooms were randomly assigned to the rearing photoschedules and birds within rooms were randomly assigned BW treatments. Individual bird was used as experimental unit.

7.3.2 Animals and housing

The experimental protocol was previously described in full detail by van der Klein et al. (2018; Chapter 5). 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 6 environmentally controlled rooms. Each room was equipped with a PF system (Zuidhof et al., 2016, 2017), which controlled individual feed intake to achieve and adhere to the assigned target

BW curves. The PF system recorded individual BW and individual feed intake for every feeding bout. Water was provided *ad libitum* with nipple drinkers during the entire experiment. From d 0 to 16, birds were fed *ad libitum* and were trained to use the PF system after which birds were tagged with a radio frequency identification (RFID) wing band. Birds were 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 birds were fed individually and were allowed access to 10 g feed for a duration of 45 s when their BW was lower than their treatment target BW. At wk 28, feed allowance was increased from 10 g to 20 g. The duration of the feed bout was maintained at 45 s throughout the study. Birds were ejected from the PF system when their measured BW was equal to or higher than their treatment target. 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 two 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. All treatments were photostimulated at wk 21 with a single abrupt increase to 16L:8D. The light source (60% red, 20% green, and 20% blue LED light bulbs; PGR-11, AgriLux, Cambridge, ON) provided light intensities of 8 lux during rearing and 25 lux during the laying phase. In each room, environmental temperature was set at 32.0°C at placement, which gradually decreased every d to 20.7°C at d 26. Environmental set temperature remained at 20.7°C throughout the remainder of the experiment. For the first 3 wk, birds received a standard wheat based starter diet (2,900 AME, 19% CP, 1.1% Ca); from wk 4 to wk 23 pullets received a wheat and barley based grower diet (2,589 AME, 14.2% CP, and 0.9% Ca); from wk 23 to wk 34 hens received a wheat based peak layer diet (2,689 AME, 15.0% CP, and 3.3% Ca); and from wk 35 to wk 55 hens received a wheat based post peak layer diet (2,682 AME 14.6% CP, and 3.3% Ca). AME, CP, and Ca values of the diets were not analyzed. At wk 18, a nest box with 8 nesting sites equipped with RFID readers was installed in each room, which identified eggs of individual hens.

7.3.3 Data collection

Birds were weighed manually on a daily basis for the first two wk to confirm growth and adoption of the PF system. After individual feeding started, the PF system recorded individual BW and feed intake on a per visit basis. Feed intake and visit frequency was checked on a daily basis to ensure all birds were accessing the PF system. Because it would not be possible for floor eggs to be linked with individual hens because hens on different BW treatments were housed in the

same room, cloacae of all hens were palpated daily to detect hard-shelled eggs in the shell gland to measure age at first egg and individual egg production from 20 wk to 36 wk. As the majority of the birds on the 8L:16D photoschedule treatment had entered lay by wk 36, from 36 wk onward, daily palpation was performed every second wk. Eggs were associated with individual hens using the RFID equipped nest box and were weighed daily. Because not all eggs could be associated with individual hens, average egg weight per BW by rearing photoperiod treatment interaction was calculated and used for EM calculations. Eggs between 40 g and 90 g were included in the calculation for average egg weight and egg mass. Average weekly BW [(BW at start of the wk + BW at the start of next wk)/2] was used for metabolic BW calculations. Average daily gain was defined as the difference between BW at start of the wk and BW at the start of the following wk, divided by 7 d. Egg production was defined as the number of eggs produced per wk divided by 7 d. For wk where individual egg production was not measured, egg production was estimated as the average of the egg production of the wk before the missing wk and the wk after the missing wk. Egg mass (EM) was defined as the product of individual egg production and the average egg weight for the individual's treatment interaction. Cumulative FCR_g was calculated as the cumulative feed intake divided by the cumulative BW gain. FCR_{egg} was calculated as the average daily feed intake divided by EM. Cumulative FCR_{egg} was calculated as the cumulative feed intake divided by the cumulative EM. Weekly averages of daily visits was recorded, in addition to weekly averages of visits at which feeding was allowed (meals), and the meal to visit (meal:visit) ratio.

7.3.4 Specification of models

Four models were evaluated: One nonlinear model, 2 nonlinear mixed models with one random term, and one nested nonlinear mixed model with two random terms (Table 7.1; based on Romero et al., 2009b). For all models, the metabolic BW scaling coefficient was allowed to fluctuate. All models included interactions between metabolic BW and ADG because requirements for gain may differ at different BW (Romero et al., 2009b). Model I was a simple nonlinear model of ME intake as a function of metabolic BW, ADG, and EM based on Byerly et al. (1980), Schulman et al. (1994), and Romero et al. (2009b). Model II was a nonlinear mixed model based on the function of model I, but included a random term $u \sim N(0,Vu)$ associated with the coefficient of metabolic BW to separate individual variation in maintenance ME from other sources of random variation. Model III was a nonlinear mixed model based on the function of model I, but included a random term $uu \sim N(0,Vuu)$ associated with the coefficient of metabolic BW by wk to separate

age variation in maintenance ME from other sources of random variation. Model IV was a nonlinear mixed model and a combination of model II and model III, including both random terms $u \sim N(0,Vu)$ and $uu \sim N(0,Vuu)$ where the age term was nested within the term of individual bird.

7.3.5 Statistical analysis

All statistical analyses were performed with SAS (Version 9.4. SAS Institute Inc., Cary, NC, 2012). The 4 models were fitted with the NLMIXED procedure, for complete code see supplementary information. Mean square errors (MSE) and R^2 were manually calculated from the estimated values using the following equations:

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

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

The linear regression between observed and estimated values of daily ME intake was conducted in the regression procedure. The analysis of BW, ADG, EM, FCR_{egg} and cumulative FCR_{egg} was conducted in the HPMIXED and MIXED procedures. The ANOVA for treatment differences in RFI, RHP, total heat production (HP), visit frequency, and meal size were conducted using the MIXED procedure. Tukey's range test was used to compare treatment means. Differences were reported where $P \leq 0.05$. The statistical ANOVA model for RHP included BW treatment and rearing photoschedule as fixed effects, and their interaction. The statistical ANOVA model for BW, ADG, EM, FCR_g, FCR_{egg}, RFI, HP, visit frequency, and meal size included BW treatment, rearing photoschedule, and age as fixed effects and all 2 and 3-way interactions. Random variation attributable to individual hens was estimated in all analyses that included serial measurements.

7.4 Results and discussion

7.4.1 Animal performance

Animal performance, including BW, BW variation, and feed intake was previously described by van der Klein et al. (2018; Chapter 5). A summary overview with treatment differences BW, ADG, and EM for both the rearing and the laying phase is provided in Table 7.2, as these were used to fit the models. The effects of the treatments on sexual maturation (van der Klein et al., 2019; Chapter 6) and reproductive performance (van der Klein et al., 2018; Chapter 5) have been discussed elsewhere. From these earlier publications it is important to highlight that treatments significantly differed in age at first egg and egg production. Of the Standard BW hens

on the 8L:16D, 10L:14D, and 12L:12D photoschedules, 3.3, 18.1, and 37.6%, respectively, never commenced egg production throughout the experiment (van der Klein et al., 2018; Chapter 5). Non-laying birds were included in the dataset used for fitting the models. For the subset of birds that were laying, productivity did not differ between treatments, hence, the difference in egg production originated from the rate (%) of hens reaching sexual maturity within each treatment. Even though age at first egg did not differ between hens under the 8L:16D and 10L:14D photoschedules (173 vs. 172 d, respectively), in the Standard BW treatment, the 12L:12D rearing photoschedule delayed age at first egg compared with the 8L:16D rearing photoperiod (266 vs. 180 d, respectively). These differences resulted in challenges comparing EM, FCR_{egg}, and cumulative FCR_{egg}, therefore data was analyzed from wk 26 onward.

7.4.2 Model bias and fit evaluation

Coefficients of model I, II, III, and IV are reported in Table 7.3-7.6, respectively. All models converged. A variation on model IV was initially attempted, where the random term associated with the individual bird was nested within the random term of age. However, this model would not converge or was unstable, depending on the starting parameters. It is hypothesized that the model would not converge because of the large variability in age at first egg between birds. As energy partitioning in birds changes from growth to egg production once birds reach sexual maturity (Leeson and Summers, 2001), birds in our population were in different physiological states at the same age. Therefore, individual bird rather than age would explain a large proportion of the differences in ME_m requirements over age.

Table 7.7 reports the results of a linear regression of the observed vs predicted daily ME intake for the four energy partitioning models. All regressions had a slope close to 1, which means that there was no change in over- or underestimation of estimates at low to high daily ME intake. For all models except model IV, the intercept was not different from 0; a systematic overestimation of 3.131 kcal/d ME intake is inferred for model IV. Figure 7.1 shows the individual residuals (RFI) of all 4 models over age. In Figure 7.1I, model I, a pattern can be observed where around 5 wk and around 15 to 25 wk residuals are larger than 0, which indicates underestimation of MEI. Adding the random term associated with individual bird did not change this pattern (Figure 7.1II), but adding the random term associated with age reduced the issue (Figure 7.1III). Adding a random term both for each individual bird and for the age of the bird, significantly reduced the overall residuals (Figure 7.1IV), and seemed to reduce the issue with bias around 15 to 25 wk. However,

the same pattern of underestimation could be seen at wk 5 as in model I and II. Standard deviation was 20.1 kcal/kg$^{0.58}$ for the random age term ($\sqrt{Vu}$, Table 7.6) and 10.9 kcal/kg$^{0.58}$ for the random individual term ($\sqrt{Vuu}$, Table 7.6) indicating twice as much variation was explained by age compared to the individual bird.

In the literature, estimates for ME_m requirements ranged from 147.6 kcal/d to 245.2 kcal/d for a 2 kg pullet or mature broiler breeder (Spratt et al., 1990; Sakomura et al., 2003; Rabello et al., 2006; Romero et al., 2009a, 2011; Hadinia et al., 2018). Estimates for the energy partitioning to maintenance for a 2 kg bird were similar for model I, II, III, and IV fell all within that range (184.1 kcal/d, 196.6 kcal/d, 179.4 kcal/d, and 195.2 kcal/d, respectively). All models also showed coefficients for the ME requirement for EM production close to values from the literature. The coefficients associated with EM were 3.14 kcal/g, 2.28 kcal/g, 3.43 kcal/g, and 2.42 kcal/g for model I, II, III, and IV, respectively. Literature reported values ranged between 1.8 kcal/g and 3.1 kcal/g (Combs, 1968; Sakomura, 2004; Romero et al., 2009b, 2011; Reyes et al., 2012; Pishnamazi et al., 2015). Model I and III estimated slightly higher values, potentially because these models did not appropriately account for sources of variation. Individuals varied substantially in egg production and unaccounted individual variation in ME_m requirements related to an increased feed intake for egg production was likely accounted for in the EM coefficient. Model III showed very different values for ME requirements for gain compared to the literature and compared to the other models. For a 2 kg bird, ME requirement per gram of gain was 1.25 kcal/g, 0.90 kcal/g, and 0.92 kcal/g for model I, II, and IV, but only 0.24 kcal/g for model III. As the exponent of BW for the requirement for gain was negative (-1.21), model III predicted a decrease in ME requirement for gain with increasing BW (Figure 7.2). This is in contrast to all other models, which predicted an increase in the ME requirement for gain with increasing BW. It was hypothesized that at higher BW (hence closer to maturity or within mature birds), more fat tissue was deposited, whereas lean mass deposition stayed relatively constant (Vignale et al., 2017). Fat tissue has a higher energy content (9.1 kcal/g) compared to lean tissue (5.5 kcal/g; Atwater, 1900). Therefore, as BW increased, the ME requirement for gain should also have increased. Model I, II, and IV coefficients are in line with this hypothesis and also approach the range of values reported in the literature: 0.71 kcal/g through 5.80 kcal/g (Pishnamazi et al., 2008, 2015; Romero et al., 2009b, 2011; Reyes et al., 2012; Hadinia et al., 2018). The literature mostly reported values associated with the mature phase only, except for Hadinia et al. (2018) at 1.52 kcal/g and Pishnamazi et al. (2008) at 0.71

kcal/g. The fact that the current models were fitted using data from both the rearing and the laying phase may have caused lower values for the ME requirement for gain. The energy requirement for gain for *ad libitum* fed broilers was previously reported at 1.15 kcal/g for females and 1.41 kcal/g for males (Romero et al., 2011), although the authors concluded that this could have been an underestimation as their model may have overestimated ME_m requirements.

All models including random terms had a better fit than model I (Table 7.8), as they showed a reduced BIC, reduced MSE, and a R^2 closer to 1. Model IV showed a significant drop in MSE and had a R^2 very close to 1, therefore, model IV was selected for further discussion of ME_m requirements and energy efficiency evaluation.

7.4.3 Maintenance energy requirements and energy efficiency

RHP and HP were evaluated using model IV and are presented separately for the rearing (< 21 wk, Table 7.9) and laying (> 20 wk, Table 7.10) phase. During the scotoperiod, HP is reduced in broiler breeders (Macleod et al., 1980) and broilers (Kim et al., 2014). Therefore, it was hypothesized that during rearing, the treatment with the shortest scotoperiod, i.e. the 12L:12D treatment, would be the least efficient and show the highest cumulative FCR_g, highest RFI, and RHP, and highest HP. The 12L:12D photoschedule treatment had indeed the highest cumulative FCR_g (2.86 g/g) and highest RFI (1.51 kcal) during rearing. However, the 12L:12D treatment also had the lowest RHP (-1.62 kcal/$kg^{0.58}$) and lowest HP (129.5 kcal/$kg^{0.58}$) during rearing. Potentially the increased photoperiod increased the level of activity in the 12L:12D treatment. The increased ME_m expenditure due to activity may have provided stimulus for a metabolic shift to become more energetically conservative with ME partitioning to HP overall in the 12L:12D treatment during rearing. During the scotoperiod melatonin secreted from the pineal gland is increased (Pang et al., 1996). Increased melatonin levels have been linked directly to improvement in feed efficiency, as they reduced energy partitioning to physical activity and therefore reduced HP in broiler chickens (Apeldoorn et al., 1999). In addition, decreased heart rate, increased blood pressure, and increased body temperature in the scotoperiod compared to the photoperiod were closely associated with energy expenditure in adult broiler breeders (Savory et al., 2006). Heart rate, blood pressure and body temperature were also lower in restricted vs *ad libitum* fed birds, except within 1 h of consuming the daily feed allotment for restricted fed birds (Savory et al., 2006), indicating that feed restriction results in a metabolic shift towards energy conservation.

Figure 7.3 shows the regression between average daily ME intake and HP summarized over the total experimental period, Figure 7.4 and 7.5 show separate regression analysis of average daily ME intake and HP for the rearing and laying phases, respectively. The slope coefficient represents the proportion of increased ME intake that was lost as heat, within the reported range, i.e. the heat increment of feeding. The model predicted that 79% of the increase in ME intake was lost as heat during the rearing phase, whereas 44% of the increase in ME intake is lost as heat during the laying phase. Hadinia et al. (2018) estimated that 87% of the increase in ME intake was lost as heat during rearing (wk 10 to 23) in broiler breeders, and Romero et al. (2011) estimated that at 65% for *ad libitum* fed broilers (wk 1 to 6). Although ME intake was not corrected for metabolic BW, Romero et al. (2009b) estimated the slope of ME intake on estimated HP between 19% and 34% during the laying phase (wk 20 to 60; depending on the model used). Both the literature and the current results indicated that a lower proportion of an increase in ME intake was lost as heat in mature birds compared to immature birds. When estimated HP in the current study was summarized based on maturity (reaching age at first egg), instead of age, 87% and 47% of the increase in ME intake was lost as heat for immature and mature birds, respectively. This suggested that the heat increment of feeding depended on the age and/or reproductive state of the bird. The results could have been confounded by dietary factors, as the diet was switched at wk 23 (from a grower to peak layer diet). However, it was previously concluded that diet composition did not affect the heat increment of feeding in broilers (van der Klein et al., submitted; Chapter 3). In immature feed restricted birds, it is possible that part of the increase in ME intake will directly partition to gain, predominantly towards lean tissues. Lean tissues are estimated to have a ten-fold higher energy requirement for maintenance compared to fat (Scott and Evans, 1992). In mature birds, an increase in ME intake partitioned to gain would mostly result in fat deposition in broiler breeders (Leeson and Summers, 2001). Therefore, the increase in HP with increased ME intake could be lower for mature birds compared to immature birds, because of a decrease in deposition of metabolically costly tissues and a relative increase in deposition of metabolically inexpensive tissues. Both BW treatment and photoschedule treatment significantly affected age at first egg (van der Klein et al., 2018; Chapter 5), therefore, an analysis was performed without accounting for treatment differences to study the differences in HP for birds in lay (mature) compared to those that had not commenced egg production (immature). Mature birds had a higher HP compared to immature birds (135.09 ± 0.35 kcal/kg$^{0.58}$ vs. 126.91 ± 0.32 kcal/kg$^{0.58}$, respectively, $P < 0.001$). The increased HP in mature birds

was likely due to an increase in feed intake to support egg production, and an obligatory increase in the heat increment of feeding.

The RHP measures energy efficiency without being confounded by feed intake, including the heat increment of feeding, BW gain, and egg production (Romero et al., 2009a). Therefore, RHP can be used as a good estimator for energy efficiency for maintenance requirements. Standard BW birds had a lower RHP compared to High BW birds during the laying phase (1.47 ± 0.643 kcal/kg$^{0.58}$ vs -1.30 ± 0.645 kcal/kg$^{0.58,}$ P = 0.003), but this difference was less clear during rearing (0.54 ± 0.434 kcal/kg$^{0.58}$ vs -0.61 ± 0.435 kcal/kg$^{0.58}$, P = 0.062). During rearing, birds might already be extremely conservative with ME utilization, because of the severe level of feed restriction. Therefore, there was little variation in RHP, i.e. they showed a RHP close to zero. RHP did not differ between hens reared under different photoschedules. It was previously concluded that adult broiler breeder hens with low RHP and low RFI produced more efficient broilers compared to broiler breeders with a low RHP and a high RFI (Romero et al., 2011). Therefore, it is suggested that future research evaluates the relationship between offspring performance for birds with differing life-time HP, RHP, and RFI, and the underlying cause of genetic or developmental differences.

The FCR_{egg} was higher for Standard BW compared to High BW birds (3.83 ± 0.07 g/g egg vs. 3.65 ± 0.05 g/g egg, P < 0.001; Table 7.10). Standard BW birds had a much lower EM compared to High BW birds (27.8 ± 0.41 g vs 42.3 ± 0.42 g, respectively; P < 0.001; Table 7.2). The lower feed intake and lower ME_m requirements in Standard BW birds did not balance out the loss in EM. FCR_{egg} and cumulative FCR_{egg} were heavily influenced by the age at first egg. BW was higher at first egg when age at first egg was delayed, therefore, ME_m requirements were higher as well, requiring a higher feed intake for the same EM. In addition, cumulative feed intake increased without an increase in EM with delayed age at first egg. This highlights that FCR_{egg} is an incomplete indicator of production efficiency for broiler breeder reproductive performance. Our results are partially congruent with results from Lewis et al. (2005). They observed that for birds reared under differing rearing photoschedules and on different BW curves, the amount of feed needed to produce 1 g of egg reduced by 0.025 g for each extra egg produced, independent from BW treatment. They also concluded that birds allowed accelerated growth were less efficient than conventionally reared birds for a given number of eggs, because of increased ME_m requirements. Similarly, the relationship between average individual FCR_{egg} and total egg production till wk 55

in the current study inferred that the decrease in FCR_{egg} with increased total egg production depended on BW treatment (-0.021 g/g for the High BW treatment and -0.040 g/g for the Standard BW treatment). FCR_{egg} of birds on the High BW treatment was higher compared to birds on the Standard BW treatment, when corrected for total egg production (analysis not shown).

7.4.4 Feeding station visit frequencies and meal size

The weekly average of daily number of visits is reported in Figure 7.6. Visiting a feeding station is a foraging-type behavior (Girard et al., 2017), therefore an increase in feeding station visits could indicate increased feed seeking motivation, which was previously linked to level of feed restriction and hunger (Dixon et al., 2014). Therefore, treatment differences in visit frequencies could be an indicator of hunger. However, no direct comparison has yet been made between visit frequency and foraging or hunger indicators currently used in the literature (behavioral or physiological). Increased visit frequency is also a measure of locomotive activity and increased locomotive activity increased ME_m requirements (van Kampen, 1976; MacLeod et al., 1982, 1988). Therefore, increased visit frequency could also be linked to increased HP (Johnson and Farrell, 1984). A linear regression between HP and daily visit frequency up to wk 21 showed that one extra visit per d corresponded to a 0.076 kcal increase in HP, after correcting for the fixed effects and interactions between BW treatment, photoschedule, and age ($P < 0.001$; $R^2 = 0.96$; results not shown).

During the rearing phase, daily visits to the feeding stations ranged between 50 to 85 times, peaking at wk 8 (Figure 7.6). Surprisingly, the 10L:14D treatment had a higher visit frequency compared to the 8L:16D and 12L:12D treatment, which aligned with a higher cumulative feed intake during the rearing period (8,260 g for the 10L:14D photoschedule vs 8,091 g for the 8L:16D photoschedule; van der Klein et al., 2018; Chapter 5) and a higher HP (Table 7.9). It is unclear why the 10L:14D treatments differed from the 8L:16D and 12L:12D treatment.

The meal:visit ratio was defined as the number of meals per d (Figure 7.7) divided by the total number of visits to the feeding station per d (Figure 7.6). The meal:visit ratio was hypothesized to be an indicator of feeding motivation. Meal:visit ratio was much lower (around 20% for all treatments) in the rearing phase compared to the laying phase (around 80%) for those treatments that commenced egg production earlier (8L:16D and High BW treatment; Figure 7.8). The meal:visit ratio was in line with results from Zuidhof (2018) who looked at Cobb grandparent pullets and found an average meal:visit ratio of 17% between wk 2 and 22. However, the meal:visit

ratio of the 12L:12D Standard BW treatment stayed around 30% in the laying phase, indicating these birds were hungrier compared to those treatments that had commenced egg production. It is hypothesized that birds that commenced egg production were less hungry as 1) treatments with high egg production had a lower overall visit frequency (Figure 7.6), 2) treatments with high egg production had a higher meal:visit ratio (i.e. they were allowed to eat around 80% of the time they visited the feeding station; Figure 7.8). In addition, every day a hen produced an egg, BW of the hen was reduced by the weight of the egg. With this BW reduction, hens qualified for additional feed allocation through the PF system, as the PF feed allocation decision was based on BW.

Birds did not restrict their visits to the PF system to the photoperiod; Standard BW birds visited the PF stations more often during the scotoperiod than the High BW birds (1.14 ± 0.01 vs 0.84 ± 0.01 times per hour). It was hypothesized that birds with shorter photoperiods, i.e. longer scotoperiods, would visit the feeding stations more often during the scotoperiod. Contrary to this hypothesis, birds with shorter photoperiod visited the stations less often during the scotoperiod (0.85 ± 0.01, 1.03 ± 0.01, and 1.10 ± 0.01 times per hour for the 8L:16D, 10L:14D, and 12L:12D photoschedules respectively, over the complete experimental period; $P < 0.001$). During the rearing phase this might have been the result of the higher energy expenditure for birds with an increased photoperiod length, which may have resulted in a higher ME_m requirement and overall energy requirement, hence a higher motivation to visit the feeding stations.

In addition to visit frequency, meal size might also be an indicator of feeding motivation. A larger meal size was related to a faster feed intake rate, as birds were limited to 45 s to finish their meal before being ejected from the feeding station. In line with the result from the visit frequency data, in wk 10, meal size was greater in the 10L:14D photoschedule treatment compared to the 8L:16D and 12L:12D photoschedule treatments (8.4 ± 0.21 g vs 6.6 ± 0.19 g and 6.0 ± 0.19 g, respectively; $P < 0.001$). At wk 28, feed allowance was increased from 10 g to 20 g and this caused an increase in meal size (Figure 7.9). The larger feed allowance elucidated treatment differences in meal size from wk 28 to the end of the study. Meal size was largest for the 12L:12D photoschedule treatment, indicating the 12L:12D photoschedule treatment had the highest feeding motivation. This is in line with the meal:visit ratio results, as birds on the 12L:12D photoschedule treatment had the lowest meal:visit ratio (Figure 7.8). However, there may have been trade-off between meal size and number of daily meals, where birds with smaller meals size could have been allowed more meals per day to fulfill their daily feed intake requirement for the associated

weight gain. Still, at wk 25 meal:visit ratio for the 12L:12D photoschedule treatment was lower compared to the 8L:16D and 10L:14D photoschedule (43 ± 2.4 % vs 66 ± 2.3 % and 56 ± 2.6 % respectively, $P < 0.001$; Figure 7.8), even though meal size was the same (6.4 ± 0.11 g; $P > 0.05$). Overall, meal size was smaller for the High BW treatment compared to the Standard BW treatment (7.38g ± 0.02 g vs 8.14 ± 0.02 g, respectively).

7.5 Conclusions

This is the first time an energy partitioning model was developed using individual data from both the rearing and the laying phase of broiler breeders housed in a free-run setting. Including random terms for both individual and age-related variation in ME_m requirements resulted in a biologically sound estimation of ME partitioning to maintenance, gain, and egg production for both the rearing and the laying phase, and reduced residuals substantially. It allowed for efficiency indicators to be estimated for the rearing and the laying phase separately, and overall. In the rearing phase, HP was related to level of egg production and therefore level of feed intake, and FCR_{egg} was confounded by age at first egg. RHP of hens on the Standard BW treatment was lower compared to hens on the High BW treatment. Age and/or reproductive status significantly affected the proportion of ME intake partitioned to HP; the slope of the regression between individual HP and ME intake was 79% during the rearing phase and decreased to 44% during the laying phase. Station visit frequency, meal:visit ratio, and meal size gave further insight into feed seeking behaviour and hunger, where birds on the 10L:14D treatment seemed to be hungriest during the rearing phase, and birds on the 12L:12D Standard treatment, with the largest proportion of non-laying birds, seemed to be hungriest during the laying phase.

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7.8 Tables

Table 7.1 Functional specifications of the evaluated models.

Model	Function specification
I	$MEI_d = a \times BW^b + c \times ADG \times BW^d + e \times EM + \varepsilon$
II[2,4]	$MEI_d = (a + u) \times BW^b + c \times ADG \times BW^d + e \times EM + \varepsilon$
III[3,4]	$MEI_d = (a + uu) \times BW^b + c \times ADG \times BW^d + e \times EM + \varepsilon.$
IV[2-4]	$MEI_d = (a + u + uu) \times BW^b + c \times ADG \times BW^d + e \times EM + \varepsilon$

[1] Estimated parameters are lowercase letters. MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADG = ADG (g/d); EM = egg mass (g/d); *u* = bird related random term; *uu* = age related random term; ε = residual error.
[2] The error term *u* was associated with each bird.
[3] The error term *uu* was associated with each age.
[4] Variances V, V*u,* and V*uu* were estimated in the regressions.

Table 7.2 BW, average daily gain (ADG), and egg mass (EM) of broiler breeder hens for the rearing (< 21 wk) and laying (> 20 wk) phase fed to achieve a High or Standard BW[1] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS).

			BW (kg)				ADG (g)				EM (g/d)	
	BW	RPS	Rearing	SEM	Laying	SEM	Rearing	SEM	Laying	SEM	EM	SEM
BW	High		1.365[a]	0.001	3.763[a]	0.002	17.3[a]	0.15	7.3	0.14	42.3[a]	0.42
	Standard		1.127[b]	0.001	3.294[b]	0.002	14.1[b]	0.15	7.2	0.14	27.8[b]	0.41
RPS		8L:16D	1.250[a]	0.001	3.489[c]	0.003	15.7	0.18	7.2	0.17	42.6[a]	0.48
		10L:14D	1.246[b]	0.001	3.520[b]	0.003	15.7	0.19	7.1	0.18	35.3[b]	0.54
		12L:12D	1.241[c]	0.001	3.577[a]	0.003	15.8	0.18	7.5	0.17	27.2[c]	0.50
BW x RPS	High	8L:16D	1.370[a]	0.001	3.709[c]	0.004	17.3[a]	0.25	7.2	0.23	45.7[a]	0.68
		10L:14D	1.367[a]	0.001	3.743[b]	0.004	17.3[a]	0.28	7.2	0.27	43.9[a]	0.80
		12L:12D	1.357[b]	0.001	3.837[a]	0.004	17.4[a]	0.25	7.6	0.24	37.3[b]	0.70
	Standard	8L:16D	1.131[c]	0.001	3.269[f]	0.004	14.1[b]	0.25	7.2	0.23	39.6[b]	0.69
		10L:14D	1.125[d]	0.001	3.297[e]	0.004	14.1[b]	0.27	6.9	0.25	26.8[c]	0.73
		12L:12D	1.124[d]	0.001	3.317[d]	0.004	14.1[b]	0.27	7.3	0.25	17.0[d]	0.73
Source of variation			P – value									
BW			< 0.001		< 0.001		< 0.001		0.42		< 0.001	
RPS			< 0.001		< 0.001		0.93		0.26		< 0.001	
BW x RPS			< 0.001		< 0.001		0.98		0.68		< 0.001	
Age			< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Age x BW			< 0.001		0.97		< 0.001		< 0.001		0.95	
Age x RPS			< 0.001		0.033		< 0.001		< 0.001		< 0.001	
Age x BW x RPS			0.96		1.00		0.8377		< 0.001		1.00	

[a-d] LSMeans within a column and treatment group lacking a common superscript differ ($P \leq 0.05$).

[1] Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

Table 7.3 Regression coefficients of nonlinear model I, describing ME partitioning to maintenance, gain, and egg production.

Coefficient	Estimate	SE	t	P > t
a	129.25	1.47	88.15	< 0.001
b	0.51	0.01	55.5	< 0.001
c	0.92	0.08	12.08	< 0.001
d	0.44	0.06	6.89	< 0.001
e	3.14	0.03	103.11	< 0.001
V	2111.44	33.83	62.42	< 0.001
Estimated equation[1]	$MEI_d = 129.25 \times BW^{0.51} + 0.92 \times ADG \times BW^{0.44} + 3.14 \times EM + \varepsilon$			

[1] MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADG = average daily gain (g/d); EM = egg mass (g/d); ε = residual error; Converged in 5 iteration calls and cpu time 0.28 s;

Table 7.4 Regression coefficients of nonlinear mixed model II describing ME partitioning to maintenance, gain, and egg production and including one random term associated with individual bird.

Coefficient	Estimate	SE	t	P > t
a	130.64	1.84	70.91	< 0.001
b	0.59	0.01	64.08	< 0.001
c	0.71	0.07	9.80	< 0.001
d	0.34	0.08	4.31	< 0.001
e	2.28	0.04	59.10	< 0.001
V	1561.64	25.28	61.77	< 0.001
V*u*	232.71	28.10	8.28	< 0.001
Estimated equation[1]	$MEI_d = (130.64 + u) \times BW^{0.59} + 0.71 \times ADG \times BW^{0.34} + 2.28 \times EM + \varepsilon$			

[1] MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADG = average daily gain (g/d); EM = egg mass (g/d); *u* = bird related random term; ε = residual error. Converged in 3 iteration calls and CPU time 1.12 s.

Table 7.5 Regression coefficients of nonlinear mixed model III describing ME partitioning to maintenance, gain, and egg production and including one random term associated with age.

Coefficient	Estimate	SE	t	P > t
a	95.45	3.30	28.94	< 0.001
b	0.91	0.03	32.88	< 0.001
c	0.56	0.11	4.99	< 0.001
d	-1.21	0.23	-5.16	< 0.001
e	3.43	0.03	116.74	< 0.001
V	1692.61	27.55	61.44	< 0.001
V*uu*	314.75	41.24	7.63	< 0.001
Estimated equation[1]	$MEI_d = (95.45 + uu) \times BW^{0.91} + 0.56 \times ADG \times BW^{-1.21} + 3.43 \times EM + \varepsilon$			

[1] MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADG = average daily gain (g/d); EM = egg mass (g/d); *uu* = age related random term; ε = residual error. Converged in 58 iteration calls and CPU time 7.06 s.

Table 7.6 Regression coefficients of nonlinear nested mixed model IV describing ME partitioning to maintenance, gain, and egg production and including two random terms associated with individual bird and age, where the age term was nested within the individual term.

Coefficient	Estimate	SE	t	P > t
a	130.57	1.15	113.80	< 0.001
b	0.58	0.01	108.23	< 0.001
c	0.63	0.03	18.05	< 0.001
d	0.54	0.06	9.76	< 0.001
e	2.42	0.04	67.10	< 0.001
V	117.79	13.58	8.68	< 0.001
V*u*	404.23	11.53	35.05	< 0.001
V*uu*	232.73	20.66	11.26	< 0.001
Estimated equation[1]	$MEI_d = (130.57 + u + uu) \times BW^{0.58} + 0.63 \times ADG \times BW^{0.54} + 2.42 \times EM + \varepsilon$			

[1] MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADG = average daily gain (g/d); EM = egg mass (g/d); *u* = bird related random term; *uu* = age related random term; ε = residual error. Converged in 26 iteration calls and CPU time 10 min and 47.84 s.

Table 7.7 Linear regression of observed (y-variable) vs estimated (x-variable) average daily ME intake for the evaluated models.

Model	Coefficient	Estimate[2]	SE	P > t[3]
I	Intercept	-1.171	1.363	0.39
	Slope	1.003	0.004	< 0.001
II	Intercept	0.455	1.135	0.69
	Slope	0.999	0.004	< 0.001
III	Intercept	0.105	1.190	0.93
	Slope	1.000	0.004	< 0.001
IV	Intercept	-3.131	0.186	< 0.001
	Slope	1.011	0.001	< 0.001

[1]Predicted values were calculated with 1 nonlinear model (I), 2 nonlinear mixed models with one random term linked with metabolic BW (associated with each individual bird (II) or age (III)), and one nested nonlinear mixed model with two random terms (IV) to describe ME partitioning to maintenance, gain, and egg production in broiler breeders.

[2]Estimated intercepts and slopes measure systematic bias of the models. Intercepts different from 0 and slopes different from 1 indicate bias.

[3]Probability indicates if the estimate differs from 0.

Table 7.8 Bayesian information criterion (BIC[1]), mean squared erorr (MSE), and R square[2] (R^2) values of the evaluated models describing ME partitioning to maintenance, gain, and egg production.

Model	Random terms associated with metabolic BW (ME_m)	BIC[1]	MSE	R^2
I	None	81,826	2111.44	0.844
II	Associated with individual bird	79,957	1531.85	0.893
III	Associated with age	80,315	1667.63	0.882
IV	Both individual bird and age	78,810	45.84	0.997

[1]Smaller values indicate a better fit of the model.
[2]Values closer to 1 indicate a better fit of the model.

Table 7.9 Cumulative feed conversion ratio for gain ($cFCR_g$), residual feed intake (RFI[1]), residual heat production (RHP[2]), and total heat production (HP[3]) of broiler breeder pullets up to 21 wk of age fed to achieve a High or Standard BW[4] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS).

	BW	RPS	$cFCR_g$ (g/g)	SEM	RFI (kcal)	SEM	RHP (kcal/$kg^{0.58}$)	SEM	HP	SEM
BW	High		2.76	0.018	0.09	0.193	0.54	0.434	137.4^{a}	0.29
	Standard		2.79	0.018	-0.16	0.196	-0.61	0.435	124.2^{b}	0.29
RPS		8L:16D	2.71^{b}	0.021	-2.03^{c}	0.228	1.85^{a}	0.509	130.7^{b}	0.34
		10L:14D	2.76^{b}	0.024	0.41^{b}	0.250	-0.34^{b}	0.562	132.2^{a}	0.38
		12L:12D	2.86^{a}	0.022	1.51^{a}	0.237	-1.62^{b}	0.524	129.5^{c}	0.34
BW x RPS	High	8L:16D	2.77^{ab}	0.030	-1.31^{c}	0.322	1.42^{a}	0.720	135.4^{b}	0.49
		10L:14D	2.66^{bc}	0.034	$-0.25b^{c}$	0.359	1.44^{a}	0.812	139.8^{a}	0.55
		12L:12D	2.85^{a}	0.030	1.4^{a}	0.323	-1.22^{b}	0.720	136.9^{b}	0.49
	Standard	8L:16D	2.64^{c}	0.030	-2.75^{d}	0.323	2.28^{a}	0.720	126.1^{c}	0.49
		10L:14D	2.86^{a}	0.033	1.08^{ab}	0.348	-2.11^{b}	0.778	124.6^{c}	0.53
		12L:12D	2.87^{a}	0.032	1.18^{a}	0.346	-2.01^{b}	0.762	122.1^{d}	0.52
Source of variation			P – value							
Age			< 0.001		< 0.001		-		< 0.001	
BW			0.24		0.36		0.062		< 0.001	
RPS			<0.001		< 0.001		< 0.001		< 0.001	
Age x BW			0.99		< 0.001		-		< 0.001	
Age x RPS			0.59		< 0.001		-		< 0.001	
BW x RPS			<0.001		< 0.001		0.015		< 0.001	
Age x BW x RPS			0.936		0.43		-		0.96	

[a-d] LSMeans within a column and treatment group lacking a common superscript differ ($P \leq 0.05$).

[1] Calculated using residuals of the nonlinear mixed model describing daily ME intake (MEI_d) as a function of metabolic BW, average daily gain (ADG), and egg mass (EM): $MEI_d = (130.57 + u + uu) \times BW^{0.58} + 0.63 \times ADG \times BW^{0.54} + 2.42 \times EM + \varepsilon$, where u is associated with each individual bird, and uu is associated with age of the individual bird, and RFI = observed MEI_d - predicted MEI_d

[2] Calculated as the residual of the regression between $a + u + uu$ and MEI for each bird: $a + u + uu = 19.83 + 0.79 \times MEI + \varepsilon$, where $a + u + uu$ = predicted total HP; ε = RHP.

[3] Calculated as $130.57 + u + uu$ from the nonlinear model described under footnote 1.

[4] Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

Table 7.10 Cumulative feed conversion ratio for egg mass (FCR_{egg}), residual feed intake (RFI[1]), residual heat production (RHP[2]), and total heat production (HP[3]) of broiler breeder hens from wk 21 to wk 55 fed to achieve a High or Standard BW[4] curve and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule (RPS).

	BW	RPS	FCR_{egg} (g/g egg)	SEM	$cFCR_{egg}$ (g/g egg)	SEM	RFI (kcal)	SEM	RHP (kcal/kg$^{0.58}$)	SEM	HP	SEM
BW	High		3.65^{b}	0.051	20.6^{b}	1.38	0.23^{a}	0.100	1.47^{a}	0.643	138.0^{a}	0.42
	Standard		3.83^{a}	0.071	40.6^{a}	1.72	-0.14^{b}	0.099	-1.30^{b}	0.645	123.0^{b}	0.42
RPS		8L:16D	3.63	0.058	14.6^{b}	1.54	0.69^{a}	0.116	-0.92	0.754	136.4^{a}	0.49
		10L:14D	3.79	0.070	13.2^{b}	1.91	0.05^{b}	0.129	0.89	0.833	132.1^{b}	0.55
		12L:12D	3.80	0.094	63.9^{a}	2.22	-0.63^{c}	0.120	0.29	0.777	122.9^{c}	0.51
BW x RPS	High	8L:16D	3.73^{ab}	0.078	9.3^{de}	2.15	0.66^{a}	0.162	0.23	1.067	140.2^{a}	0.69
		10L:14D	3.76^{ab}	0.092	7.2^{e}	2.55	0.52^{a}	0.190	3.70	1.204	143.2^{a}	0.81
		12L:12D	3.46^{b}	0.093	45.3^{b}	2.45	-0.52^{b}	0.166	0.49	1.067	130.7^{b}	0.70
	Standard	8L:16D	3.53^{b}	0.085	20.0^{c}	2.20	0.73^{a}	0.164	-2.06	1.067	132.6^{b}	0.70
		10L:14D	3.82^{ab}	0.106	19.2^{cd}	2.86	-0.41^{b}	0.175	-1.91	1.152	121.1^{c}	0.74
		12L:12D	4.14^{a}	0.164	82.5^{a}	3.71	-0.74^{b}	0.174	0.09	1.129	115.2^{d}	0.74

Source of variation	P – value				
Age	< 0.001	< 0.001	< 0.001	-	< 0.001
BW	0.040	< 0.001	0.011	0.003	< 0.001
RPS	0.111	< 0.001	< 0.001	0.25	< 0.001
Age x BW	0.014	0.86	< 0.001	-	0.077
Age x RPS	< 0.001	0.73	< 0.001	-	< 0.001
BW x RPS	< 0.001	< 0.001	0.013	0.073	< 0.001
Age x BW x RPS	0.41	0.98	0.022	-	0.68

[a-d] LSMeans within a column and treatment group lacking a common superscript differ ($P \leq 0.05$).

[1] Calculated using residuals of the nonlinear mixed model describing daily ME intake (MEI_d) as a function of metabolic BW, average daily gain (ADG), and egg mass (EM): $MEI_d = (130.57 + u + uu) \times BW^{0.58} + 0.63 \times ADG \times BW^{0.54} + 2.42 \times EM + \varepsilon$ and RFI = observed MEI_d - predicted MEI_d

[2] Calculated as the residual of the regression between $a + u + uu$ and MEI for each bird: $a + u + uu = 55.30 + 0.44 \times MEI + \varepsilon$, where $a + u + uu$ = predicted total HP; ε = RHP.

[3]Calculated as $130.57 + u + uu$ from the nonlinear model described under footnote 1.

[4]Hens followed either the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High).

7.9 Figures

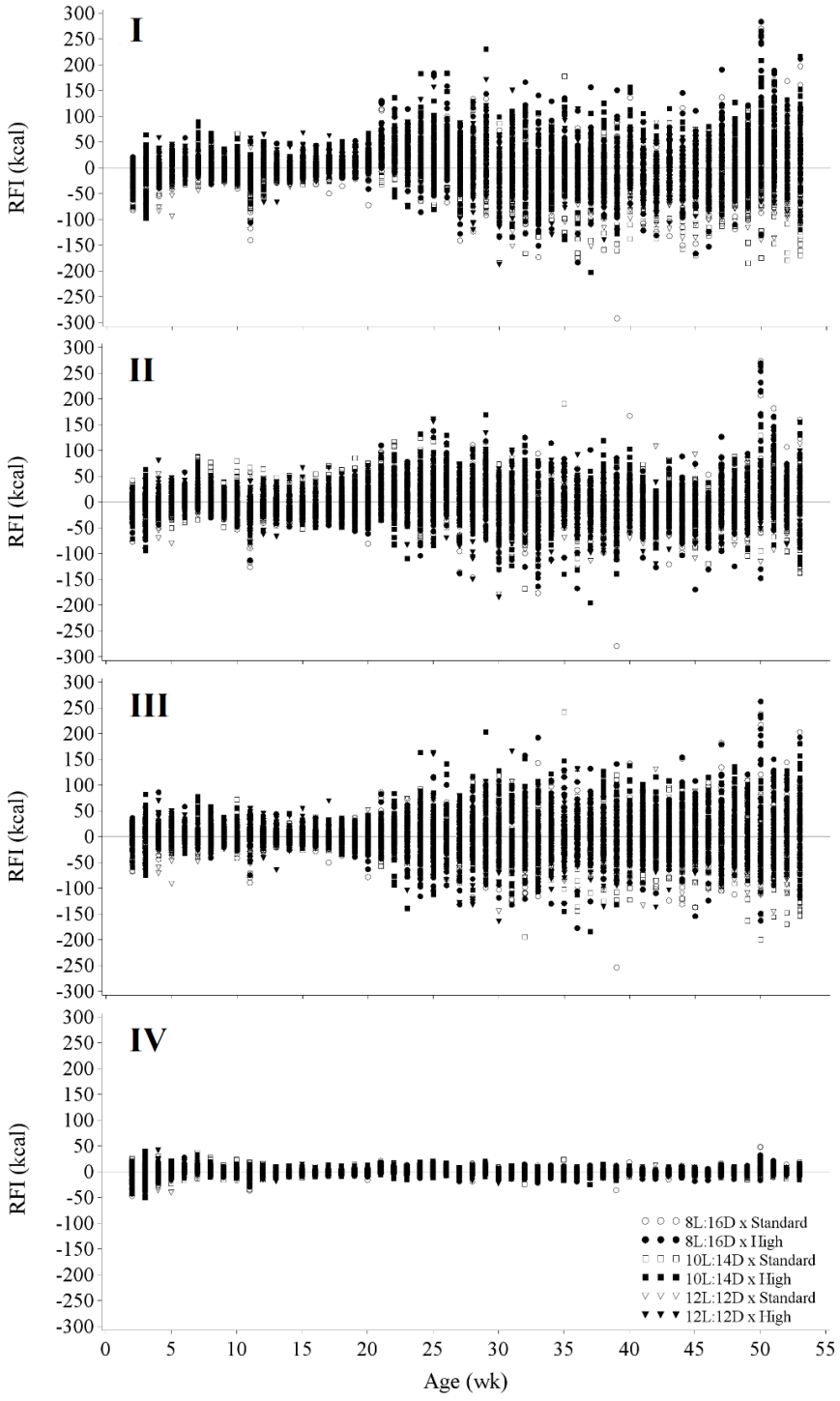

Figure 7.1 Residual feed intake (RFI) estimated from 2 to 54 wk of age for individual birds with a nonlinear model (I), 2 nonlinear mixed models with one random term linked with metabolic BW (associated with each individual (II) or age (III)), and a nonlinear mixed model with two nested random term (IV) to describe ME partitioning to maintenance, gain, and egg production.

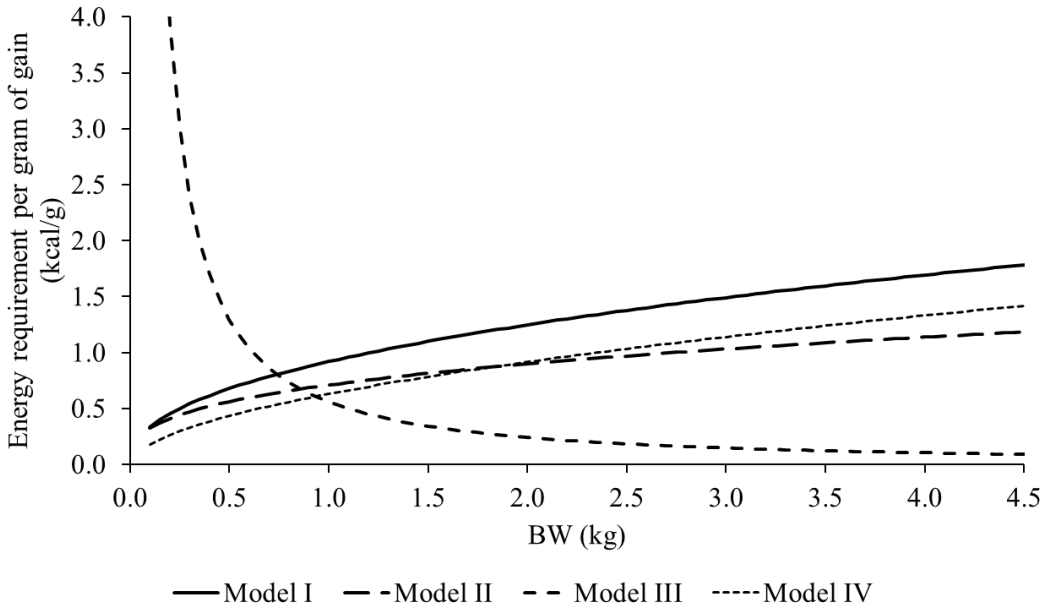

Figure 7.2 Energy requirement per gram of average daily gain (ADG) as a function of BW estimated by four models explaining average daily ME intake as a function of metabolic BW, gain, and egg mass.

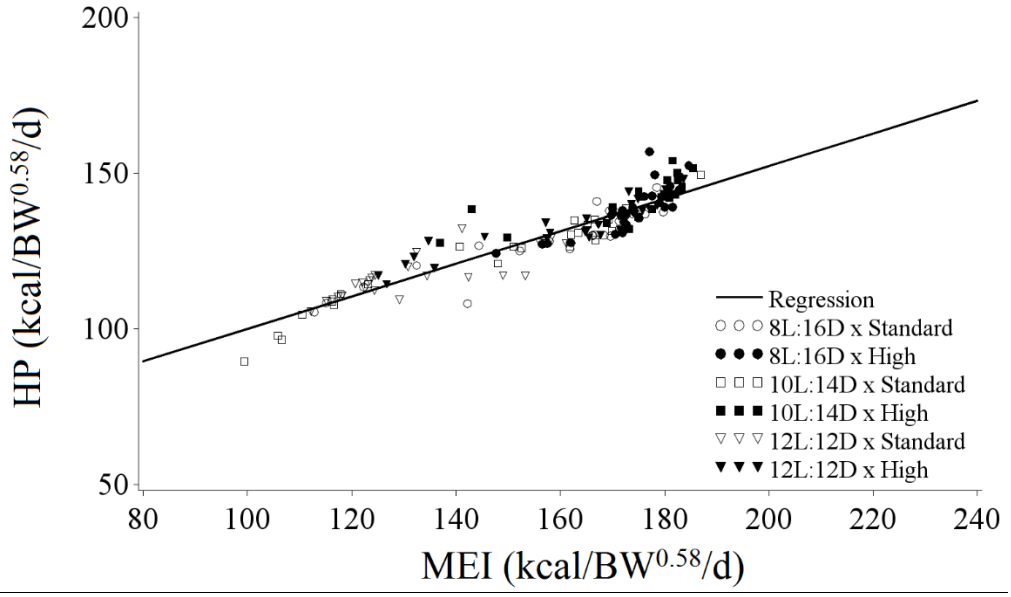

Figure 7.3 Total heat production (HP) relative to average daily ME intake (MEI) for the duration of the experiment (wk 2 to 55) as estimated by a model describing ME partitioning to maintenance, gain, and egg production and including two random terms associated with individual bird and age (IV). Broiler breeders were fed to achieve 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. Regression equation was HP = 47.74 + 0.52 × MEI + ε ($P < 0.001$; $R^2 = 0.86$)

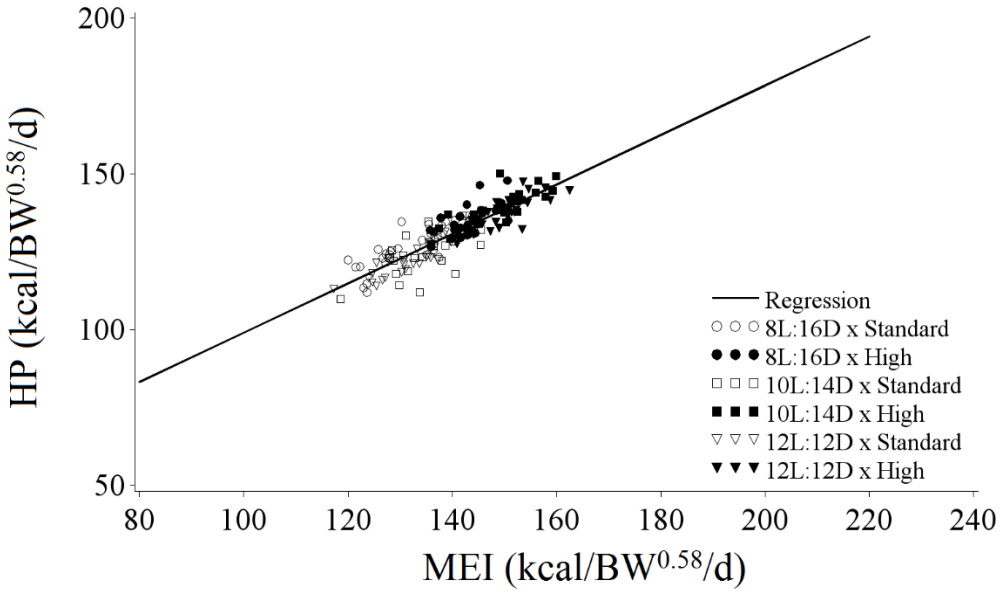

Figure 7.4 Total heat production (HP) relative to average daily ME intake (MEI) during the rearing phase (wk 2 to wk 21) as estimated by a model describing ME partitioning to maintenance, gain, and egg production and including two random terms associated with individual bird and age (IV). Broiler breeders were fed to achieve 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. Regression equation was $HP = 19.83 + 0.79 \times MEI + \varepsilon$ ($P < 0.001$; $R^2 = 0.78$).

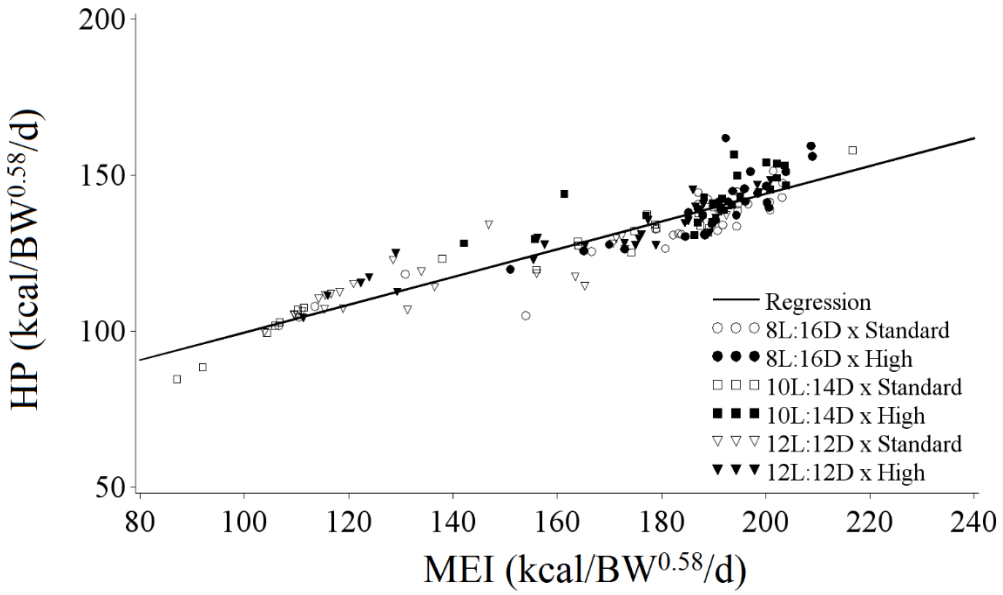

Figure 7.5 Total heat production (HP) relative to average daily ME intake (MEI) during the laying phase (wk 21 to wk 55) as estimated by a model describing ME partitioning to maintenance, gain, and egg production and including two random terms associated with individual bird and age (IV). Broiler breeders were fed to achieve 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. Regression equation was $HP = 55.30 + 0.44 \times MEI + \varepsilon$ ($P < 0.001$; $R^2 = 0.86$)

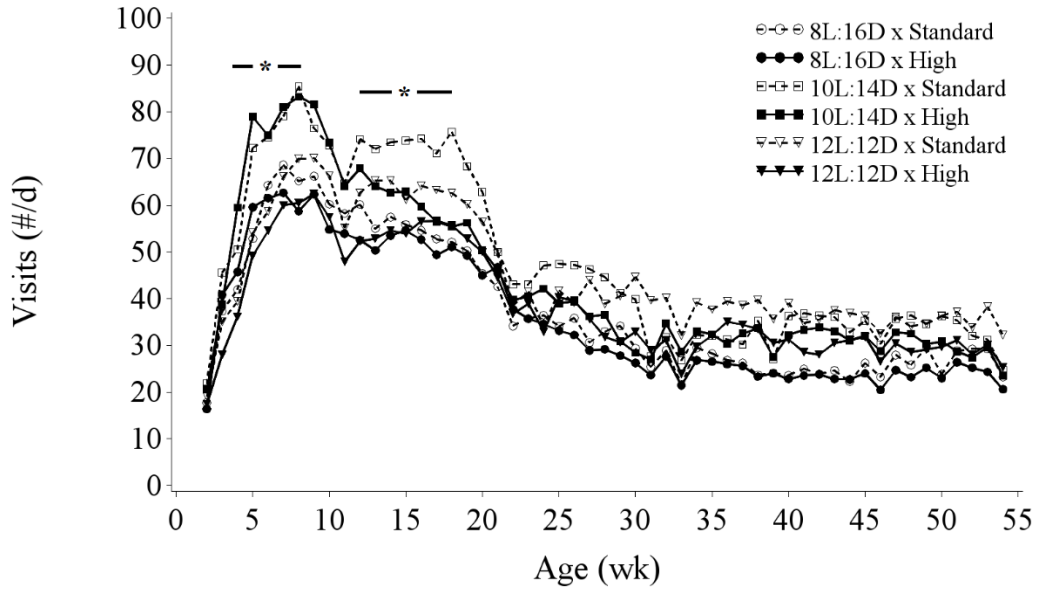

Figure 7.6 Weekly average of daily visits to the feeding station for broiler breeders fed to achieve the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule. The asterisks indicate ages where treatment means differed ($P < 0.05$); all fixed effects and interactions were significant ($P < 0.001$), except the interaction between age and BW treatment ($P = 0.08$) and the 3-way interaction between age, BW treatment, and photoschedule ($P = 0.99$).

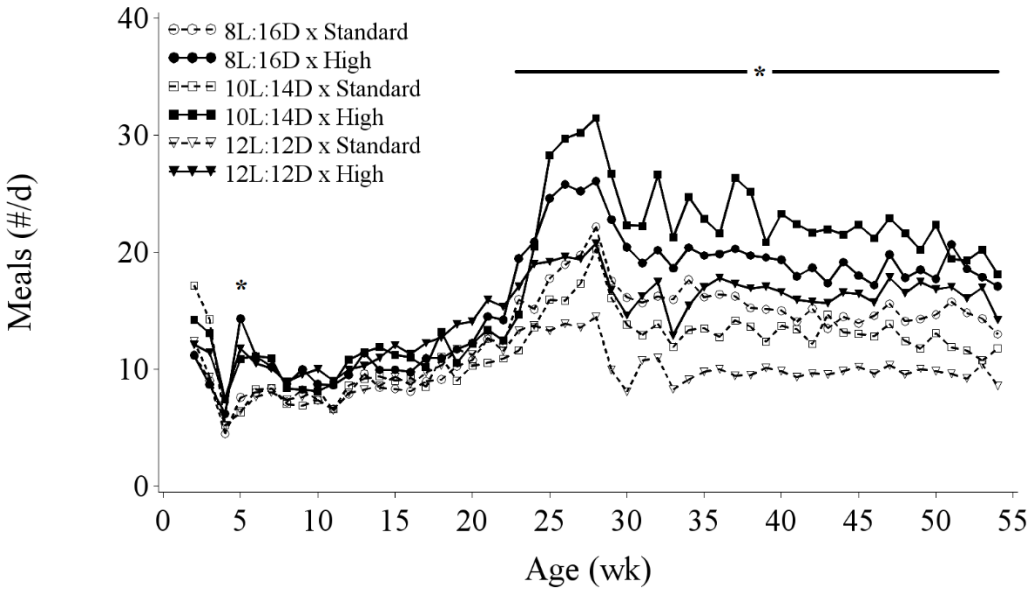

Figure 7.7 Number of daily meals of broiler breeders fed with a precision feeding system to achieve the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule. The asterisks indicate ages where treatment means differed ($P < 0.05$); all fixed effects and interactions were significant ($P < 0.001$).

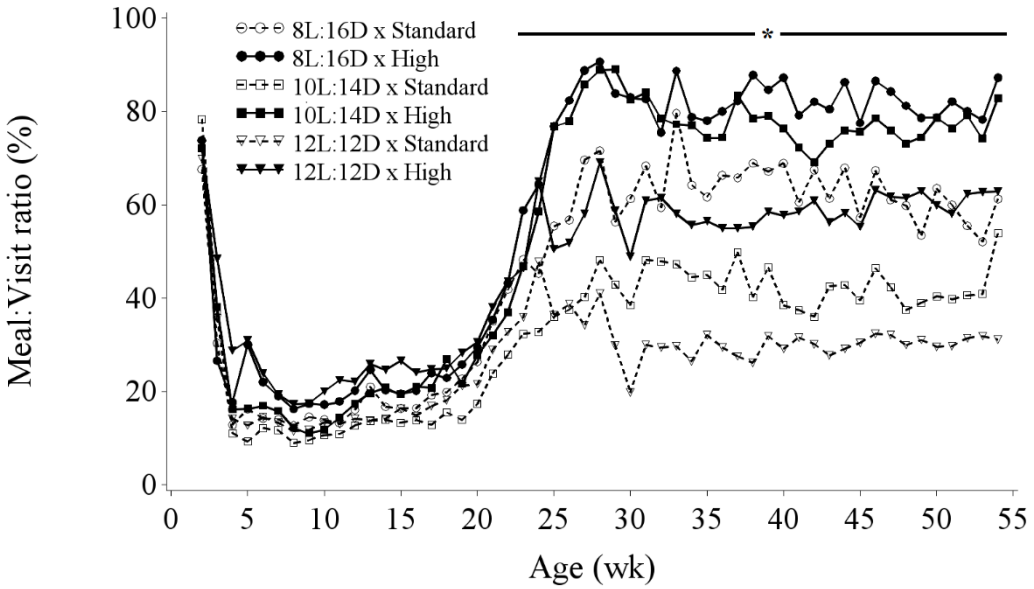

Figure 7.8 Meal:visit ratio of broiler breeders fed with a precision feeding system to achieve the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule. The asterisks indicate ages where treatment means differed ($P < 0.05$); all fixed effects and interactions were significant ($P < 0.001$), except the 3-way interaction between age, BW treatment, and photoschedule ($P = 0.07$).

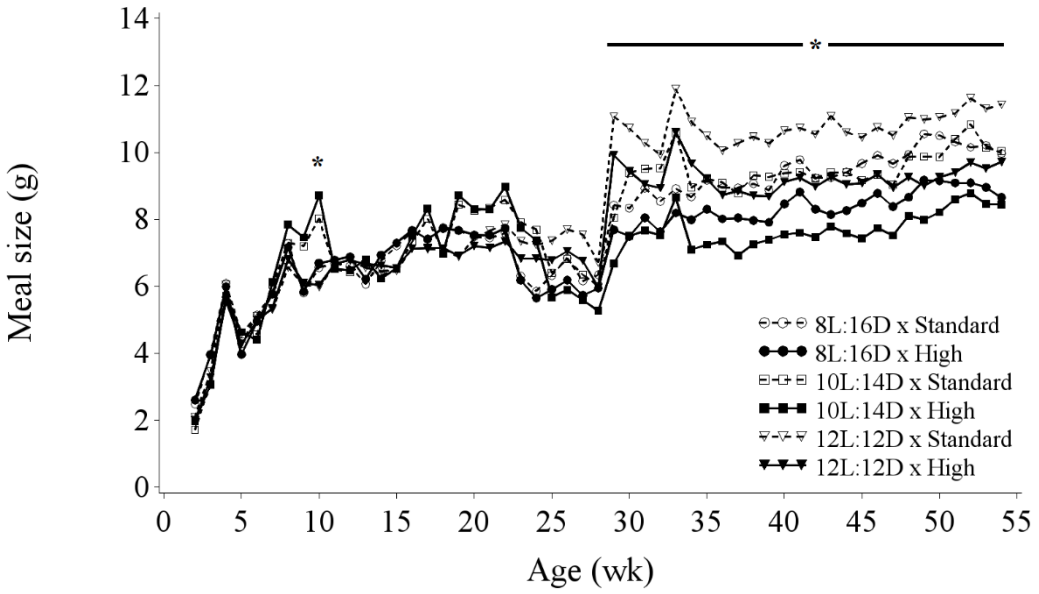

Figure 7.9 Meal size of broiler breeders fed with a precision feeding system to achieve the breeder-recommended BW curve (Standard) or an accelerated BW curve reaching the 21 wk BW at 18 wk (High) and reared to wk 21 on an 8L:16D, 10L:14D, or 12L:12D photoschedule. The asterisks indicate ages where treatment means differed ($P < 0.05$); all fixed effects and interactions were significant ($P < 0.001$), except the 3-way interaction between age, BW treatment, and photoschedule ($P = 1.00$).

7.10 Supplementary information

```
MODEL I
proc nlmixed data=[dataset];
parms      a=129.25
      b= 0.5124
      c=0.9216
      d= 0.4393
      e=3.1410
      vare= 2111.44;
pred= (a)*bwkgmid**b + c*adg*bwkgmid**d+ e*EM;
model admei ~ normal(pred, vare);
      bounds a > 0;
      bounds b > 0;
      bounds c > 0;
      bounds vare > 0;
predict pred out=pred der;
predict admei-pred out=resid ;
ods output parameterestimates=sol;
run;
quit;

MODEL II
proc nlmixed data=[dataset];
parms      a=130.64
      b= 0.5908
      c=0.7062
      d=0.3391
      e=2.2778
      vare= 1561.64
     varu= 232.71;
pred= (a+u)*bwkgmid**b + c*adg*bwkgmid**d+ e*EM ;
model admei ~ normal(pred, vare);
random u~normal(0, varu) subject=rfid;
      bounds a > 0;
      bounds b > 0;
      bounds c > 0;
      bounds varu >0;
      bounds vare >0;
predict pred out=pred;
predict a+u out=au;
predict u out = u;
predict (a + u)* bwkgmid**b out = energyMEm;
predict admei/(bwkgmid**b) out = relMEintake;
predict c*adgp*bwkgmid**d out = energygain;
predict e*EM out = energyegg;
predict admei-pred out=resid ;
ods output parameterestimates=sol;
```

```
run;
quit;

MODEL III
proc nlmixed data=[dataset];
parms      a=  93.0027
           b=   0.9571
           c=  0.4078
           d=  -1.5287
           e=  3.4310
           vare= 1 1000
      varuu= 1 100
;
pred= (a+uu)*bwkgmid**b + c*adg*bwkgmid**d+ e*EM ;
model admei ~ normal(pred, vare);
random uu~normal(0, varuu) subject=week;
      bounds a > 0;
      bounds b > 0;
      bounds c > 0;
      bounds varuu > 0;
      bounds vare > 0;
predict pred out=pred;
predict a+uu out=auu;
predict uu out = uu;
predict (a + uu)* bwkgmid**b out = energyMEm;
predict admei/(bwkgmid**b) out = relMEintake;
predict c*adgp*bwkgmid**d out = energygain;
predict e*EM out = energyegg;
predict admei-pred out=resid ;
ods output parameterestimates=sol;
run;
quit;

MODEL IV
proc nlmixed data=[dataset];
parms
      a= 130.61
      b= 0.5799
      c= 0.6242
      d= 0.5421
      e= 2.4221
      varu= 100
     varuu= 100
      vare= 100;
pred= (a+u+uu)*bwkgmid**b + c*adg*bwkgmid**d+ e*EM ;
model admei ~ normal(pred, vare);
random u~normal(0, varu) subject=rfid;
```

```
random uu ~ normal(0,varuu) subject = week(rfid);
     bounds a >0;
     bounds b > 0;
     bounds varu > 0;
     bounds varuu > 0;
     bounds vare > 0;
predict pred out=pred der;
predict a+u+uu out = auuu;
predict a+uu out=auu;
predict a+u out = au;
predict uu out = uu;
predict u out = u;
predict (a + uu + u)* bwkgmid**b out = energyMEm;
predict admei/(bwkgmid**b) out = relMEintake;
predict c*adgp*bwkgmid**d out = energygain;
predict e*EM out = energyegg;
predict admei-pred out=resid4;
ods output parameterestimates=sol;
run;
quit;
```

CHAPTER 8.

Synthesis

8.1 General discussion

8.1.1 Energy partitioning

The objective of this thesis was to understand the effects of controlling energy intake and photoperiod on energy balance between maintenance, growth, and reproduction in meat-type chickens. Reducing ME_m requirements by means of energy restriction, diet composition, or lighting schedule was hypothesized to release ME for growth and reproduction, hence optimize production efficiency. ME_m is the sum of all heat losses and includes, amongst others, the heat increment of feeding (NRC, 1981). Providing a high ME diet to broilers or moderately increasing ME intake as compared to the industry standard in broiler breeders did not alter the heat increment of feeding as measured through the linear regression of ME intake on HP (Chapter 3 and Chapter 7). In broilers, the heat increment of feeding was estimated at 26% of MEI (Chapter 3), whereas for broiler breeders this was estimated at 79% during the rearing phase and at 44% during the laying phase (Chapter 7). The difference between the estimates between these meat-type chickens could be explained by the large difference in feed allocation; in Chapter 3 these ranged between 50% of *ad libitum* to *ad libitum*, but in Chapter 7 all pullets were restricted to approximately 20 to 25% of *ad libitum* feed intake during rearing (Aviagen, 2016, 2019). It was hypothesized that reaching sexual maturity would reduce the heat increment of feeding, because of the change in energy prioritization from growth to egg production (Yu et al., 1992); any increase in MEI would be partitioned mainly to egg production during the mature phase. During rearing, any increase in MEI was hypothesized to be partitioned to lean growth, of which a large proportion resulted in additional ME_m requirements and heat production (Muramatsu et al., 1987; Scott and Evans, 1992). However, the assumption of the energy partitioning models was that ME_m requirement and the ME partitioning to gain are independent (Birkett and de Lange, 2001; Romero et al., 2009). Because of differences in the form of the retained energy, fat vs. protein, the composition of gain should impact the ME cost for that gain. For example, ME requirements for gain were estimated at 1.09 $kcal/kg^{-0.18}/g^{1.19}$ for broilers, equivalent to 2.61 kcal/g for a 1 kg broiler growing 100 g (Chapter 3), and 0.63 $kcal/kg^{0.54}/g$ for broiler breeders, equivalent to 0.63 kcal/g for a 1 kg broiler breeder (Chapter 7). In this thesis, the broilers had a higher fat pad weight compared to the breeders (14.2 g for broilers fed the Low ME diet and 21.0 g for broilers fed the High ME diet at d 42,

Chapter 3, vs 10 g for the broiler breeders on the High BW target and 5.5 g for broiler breeders on the Standard BW target at wk 54, Chapter 5). The energy expenditure of fat was less than one tenth that of liver and muscle (Scott and Evans, 1992) and protein synthesis was estimated to range between 14 and 21% of the basal metabolic rate in broilers at d 14 (Muramatsu et al., 1987). Therefore, increased lean tissue deposition could lead to reduced ME cost for gain and higher ME_m requirement due the higher maintenance costs for lean tissue. In addition, ME_m requirements were also increased with increased lean tissue deposition, because the efficiency for protein deposition was only 48%, which was much lower than the efficiency of fat deposition at 82% (Leeson and Summers, 2001). The ME used for product formation was incorporated in the ME_m requirement (NRC, 1981), therefore, more heat was lost for protein deposition as compared to fat deposition. Therefore, the assumption that ME_m requirements and ME cost for gain were fully independent was not valid. New models including the interaction between ME_m requirements and ME cost for gain and the composition of gain could be worth investigating as they may increase the understanding of energy partitioning.

8.1.2 Precision feeding and egg production

Managing broiler breeder pullets has become challenging, because feed allocation has significantly reduced relative to the pullets' genetic growth potential (Renema et al., 2007b). This has led to increased competition for feeder space and feed, resulting in reduced BW uniformity. The genetic selection process has delayed the timing of photostimulation and increased the length of the rearing period (Renema et al., 2001, 2007a), hence prolonging the period without revenue for hatching egg producers. In a free market, producers would be able to extend the laying cycle, yet in the Canadian supply managed production system this is currently not an option. The ability of the PF system to individually control BW and feed intake provides an opportunity to manage BW of broiler breeders extremely accurately. It was hypothesized that using the PF system the rearing period could be reduced by advancing the growth target and photostimulating earlier, given a BW CV of less than 2% (Zuidhof et al., 2017). Although photostimulating earlier at a high BW target allowed some pullets to advance their sexual maturation, a significant proportion of birds had not dissipated the photorefractory state, did not respond to photostimulation, and delayed their sexual maturation beyond that of precision fed birds photostimulated at a conventional age at the same weight (Chapter 4). Hence, evidence was provided to reject the hypothesis that the rearing period could be shortened using the PF system. An explanation could be that the PF system limited

birds with a higher mature BW target than provided by the breeder recommendations. Under conventional feeding circumstances, these birds would have been able to increase their growth above the target, as their higher motivation to eat would lead them to compete more ferociously for feeding space and increase their rate of feed intake, which may induced hormonal changes as well.

8.1.3 Interaction between photoperiod and body weight

Hatching egg producers in Colombia rear broiler breeders in open sided housing, where natural daylight provides rearing photoperiods around 12L:12D, much longer than the recommended 8L:16D (Lewis, 2006). However, production levels in Colombia are close to the genetic potential of the bird. It was thought that the extensive grading practices would mitigate the effect of a slower dissipation of the photorefractory state. These grading practices are available to Colombian producers because of cheap labour costs. Therefore, it was hypothesized that rearing photoperiod could be increased when BW variation was reduced with the PF system. This would also provide the opportunity to optimize the use of the PF system, as chickens prefer to eat during the photoperiod hours (Savory, 1976). In addition, it was hypothesized that BW variation could have confounded previous results on the study of the interaction between rearing photoperiod and BW (Lewis, 2006). In Chapter 5 it was discussed that the photoperiod should not be increased over the recommended length of 8 h during rearing for broiler breeders, even if BW variation is reduced to a minimum using the PF system. However, in accordance with our initial hypothesis, BW variation may indeed have confounded results on the effects of photoperiod in previous studies (Lewis, 2006). The effects of rearing photoschedules on reproductive performance depended on target BW and increasing the BW target limited the detrimental effects of increasing rearing photophotoperiod on reproductive performance. Hatching egg producers in Colombia may use increased BW targets to obtain the observed production levels.

8.1.4 Endocrinology of sexual maturation

In Chapter 6 part of the underlying endocrinological cause of this interaction was discussed. Estradiol-17β levels were measured, as this is one of the main reproductive hormones initiating sexual maturation (Deeley et al., 1975; Wilson and Sharp, 1976; Etches, 1990). Modeling was used as a tool to quantify treatment differences in E2 dynamics. After photostimulation, the start of small follicle development results in an increase in circulatory E2 levels (Renema et al., 2001), resulting in physiological changes needed for the onset of lay (Deeley et al., 1975; Etches,

1990). Quantitatively comparing both the timing of the E2 increase relative to 1) the moment of photostimulation or 2) the age at first egg provided insight as to whether there were treatment differences in 1) ovary responsiveness to produce E2 or 2) the ovary and hypothalamic feedback loop for onset of ovulation and egg production. The models based on physiological age concluded that the difference in the age at first egg originated from differences in the timing of the E2 increase relative to photostimulation (i.e. the responsiveness of the ovary). The timing of the E2 increase relative to the age at first egg only differed approximately one day between the 10L:14D and 8L:16D rearing photoschedule treatments, independent from BW. As this was the first time E2 dynamics were quantitatively compared, the underlying cause was unknown. Deep brain photoreceptors around the hypothalamus collect environmental information on lighting (wavelength, duration, and intensity) for reproduction (Bédécarrats et al., 2016). The inability of some hens to respond to photostimulation with an increase in E2, either as a result of rearing photoschedule or BW treatment, was a sign that one of the endocrinological mechanisms between deep brain photoreceptors and the hypothalamus-pituitary-gonadal axis was missing. This missing component could exist between the deep brain photoreceptors and the hypothalamus, between the hypothalamus and the pituitary, or between the pituitary and the ovary, and either a signalling hormone or their receptor might be inactivated or absent. Apart from circulatory levels of luteinizing hormone (LH) and follicle stimulation hormone (FSH), gene expression patterns of gonadotropin releasing hormone (GnRH-I), GnRH receptor I and III (GnRH-RI, GnRH-III), gonadotropin inhibitory hormone (GnIH) and GNIH receptor (GnIH-R) within the hypothalamus and pituitary would need to be investigated to find the missing mechanism. With regard to the difference in BW, Hadinia (2019) concluded that a increased ME intake after photostimulation increased the expression of GnRH-I (hypothalamus), GnRH-RI (pituitary), and LH (pituitary), increased circulatory levels of LH, FSH, and E2, and advanced sexual maturation in broiler breeder pullets. This suggested that either signalling between the deep brain photoreceptors and the GnRH pathway could be missing, or receptors could be absent or inactivate when ME intake is insufficient. However, it is unclear what level of ME intake would be insufficient, and whether this would be the case for birds reared under long photoperiods, which would have inadequately dissipated the photorefractory state. The general conclusion was that broiler breeders are still heavily dependent on short photoperiods during rearing and that the timing of photostimulation is critical for the ability to respond to the increased photoperiod with sexual maturation. However,

in contrast to previous conclusions, sexual maturation and egg production are affected by the interaction between BW target and rearing photoschedule.

8.2 Novelty of presented research

This thesis compared for the first time the standard comparative slaughter technique with a modeling methodology for determining NE_g values for feed (Chapter 3). This was the first time the PF system was used for broilers and paired feeding was implemented through the software of the PF system. Although paired feeding has been implemented in several other studies (e.g. Siegel and Wisman, 1966, O'sullivan et al., 1992), it was never done so automated and in real time. In past paired feeding studies, restricted feeding was delayed because *ad libitum* feed intake needed to be measured first, before the proportional feed intake could be calculated and weighed. Because manual feed intake measurements are labour intensive, this is often done on a daily basis at most. As a result, feed allocation frequency was also on a daily basis. The potential confounding factor of feed allocation frequency was reduced with the use of the real-time paired feeding in the PF system, as meals of restricted pair-fed birds were dispersed over the day and closer commercial practices in which feed intake would be compromised. The intent of paired feeding in Chapter 3 was providing a wide range of differing feed intakes to individuals fed one of two different diets, such that regression analysis could be used. The automated way of paired feeding ensured accurate and precise feed allocation relative to real time *ad libitum* intake.

The results from the first life-time experiment for broiler breeders fed with the PF system were described in Chapter 4 to 7. It was found that a significant interaction between BW and rearing photoschedule affected age at sexual maturity and egg production (Chapter 4), which contradicted conclusions from previous studies. It was hypothesized that this was the result of more accurate and precise control of BW and feed allocation by the PF system, which reduced BW variation. A modeling methodology was presented for endocrinological data which allowed for the first time to quantitatively compare dynamics of endocrinological results over time (Chapter 6). This modeling methodology supported an increased holistic understanding of poultry endocrinology. A novel energy partitioning modeling methodology was also presented which included both random variables for individual as well as age-related ME_m requirements (Chapter 7). This provided insight into additional variables affecting energy partitioning in poultry and provided a tool to study differences between individuals. It exemplified how advances in computing methods and computing power could enhance the understanding of energy partitioning.

8.3 Study limitations

The PF system developed at the University of Alberta (Zuidhof et al., 2018) is one of the most advanced research tools in poultry science, however, it also has limitations with regard to translation of the results to the industry. Hatching egg producers may rely on skip-a-day feeding during rearing, and once-a-day feeding during laying, hence, they apply low feeding frequencies. It was indicated in Chapter 4 and 5 that the poor performance of the hens on the breeder recommended BW treatment could have been due to an unintended effect of increased feeding frequency on metabolism and body composition, potentially reducing body lipid stores.

Implementing new treatment strategies with the PF system also resulted in unintended sensitivities in the system. The pair feeding strategy used in Chapter 3 paired one individual 'lead' bird fed *ad libitum* with individual feed restricted 'follow' birds. The sensitivity of this approach was that when the lead bird was not doing well, or needed to be culled, the follow birds needed to be paired with a new lead bird, which may alter their level of restriction. In one instance during the experiment described in Chapter 3 the initially assigned lead bird for a group of follow birds had to be altered at the end of the experiment. In new versions of the software this issue was eliminated through calculating the feed restriction level from a group of *ad libitum* fed lead birds. Throughout the length of the study in Chapter 4-7, software changes were implemented. The majority of these changes were extremely beneficial and saved labour input or improved data integrity. An example would be the ability to tare and calibrate the scales within the PF system with a click of a button, instead of manually calculating and inputting slopes and intercepts for tare and calibration purposes. The effect of the skill of hens to trick the scales of the system by scratching or jumping was also minimized due to changes in the algorithm used to measure the BW for feed allocation decisions. Other changes, often related to background working of the software, could have benefited from some rigorous testing first, as errors sometimes occurred that restricted the functionality of the PF system and resulted in downtime. Most changes to the software improved the data integrity and quality of the research overall. However, the study described in Chapter 7 specifically aimed at studying changes in ME_m requirements throughout the experimental period, therefore, some of the results might have been partially influenced by software changes. Changing the PF system's software during an experiment is not recommended, unless the changes are tested rigorously and do not affect the experimental treatments.

Hardware issues can also influence the success of an experiment using the PF system. Due to logistical reasons, the experiments in this thesis only used 1 PF station per pen of birds. Although the capacity of each station can go up to at least 30 birds per station, it is recommended that more than 1 PF station is positioned in each pen, irrespective of the number of birds in each pen. Birds do not have access to feed if the hardware of the only one available station fails. Although the stations in the experiments in this thesis were managed extremely closely, there were periods of time birds did not have access to a working PF station. This would have been resolved if 2 stations per pen were available. In more recent studies, this has been addressed by placing more than 1 PF station per pen.

In all studies measuring feed intake, results can be skewed by spilling of feed by the birds. Most spilled feed fell from the feed trough on the feed spill collection tray inside the PF system, which accumulated over a period of 24 h to up to 1 kg per PF station in the broiler experiment (Chapter 3) and at the start of the broiler breeder experiment. However, it was unknown whether this spillage originated from spillage by the birds (measured as intake), or from spillage during the filling of the feed trough between feed bouts (not measured as feed intake). About 5 months into the broiler breeder experiment described in Chapter 4 to 7, a hardware change almost eliminated feed spillage. A so-called 'feed skirt' was added over top of the feed trough, which reduced the spillage to less than 1 g of feed dust per day per PF system. However, placing the feed skirts could have artificially decreased the measured feed intake, as the portion of feed spillage by the bird would have been reduced.

The reality of many hatching egg producers in Canada is that male and female chicks need to travel by truck from the primary breeder in the southern part of the United States. These lengthy transport times, in addition to wait times at border control between the United States and Canada, led to very poor chick quality in the studies in Chapter 4 to 7. One chick was dead on arrival and 40 female chicks (over 16%) died or had to be culled within the first 2 weeks. As early life experience and stress can have long term consequences in mammals and birds, it is possible that the results may have been influenced by the early life high stress environment related to gut development and metabolism.

During the laying period of the experiment in Chapter 4 to 7, there was a high incidence of floor eggs. This resulted in the need for cloacal palpation throughout the experiment and limited available data on egg weights, as few eggs could be associated with individual birds through the

RFID equipped nestboxes. In addition, the LED light bulbs used in the experiment in Chapter 4 to 7 were originally designed for laying hens and contained a significant proportion of red lights. As this was the first time using these light bulbs for broiler breeders, the intensity of the bulbs was only adjusted to breeder recommendations such that the effects of intensity and photoperiod would not be confounded. However, increasing the light intensity could have reduced the number of floor eggs.

8.4 Future research directions

This thesis answered some of the originally defined questions in Chapter 2, however, these answers also led to new questions. Most of the newly formed questions originated from the fact that the hens on the breeder recommended BW treatment in Chapter 4 to 7 performed poorly. If the BW target curve was recommended by breeder guidelines, why would they not provide optimal results? Simultaneously with the experiment in Chapter 4 to 7, Dr Zuidhof performed an experiment comparing the life-time productivity of Cobb broiler breeder grandparent hens. The results were described in Zuidhof (2018) and provided insight on what could have been the origin of the failure of some of the birds to commence egg production. The latter study concluded that poor reproductive performance of precision fed birds could originate from altered metabolism and body composition as a result of increased feeding frequency. Low feeding frequency (i.e. conventional feeding) forces birds go through a cycle of a short feeding time and long fasting time, which leads a feasting metabolic state of digestion, absorption, and storage of nutrients (lipid deposition) alternating with a fasting metabolic state in which the stored nutrients are used to supply the nutrient requirements of the birds (Zuidhof, 2018; Hadinia, 2019). Even though both Standard BW and High BW birds were precision fed, the increased feed allowance in High BW birds may have increased lipid deposition and, therefore, the High BW birds would have been better prepared for egg production compared to Standard BW birds. A pilot experiment performed by Dr Zuidhof confirmed that birds continuously fed towards a breeder recommended BW had lower abdominal fat pad weights compared to birds fed for only 4 h/d towards the same target at 30 wk (Zuidhof, unpublished results). However, results from precision feeding studies from Afrouziyeh and Zuidhof (2019) concluded that there was no effect of a 10% increase in target BW at photostimulation in precision fed birds on age at first egg. In addition, age at first egg was in line with breeder performance guidelines in their studies. There may have been an effect of a smaller sample size in the latter experiment, which did not provide the statistical power to detect

differences, as numerical differences were present. In addition, a more aggressive increase in light intensity at photostimulation (to around 60 lux) was used to prevent floor eggs, which may have photostimulated the birds more aggressively as compared the experiment in Chapter 4 to 7. Chick quality of the breeder pullets was also better compared to chicks in the current thesis. Hence, this all still left the question unanswered, does increasing feeding frequency through precision feeding alter the birds' metabolism such that the development of sexual maturity is hampered, and if so, how?

The more general unanswered question is: when are broiler breeders ready to initiate the process sexual maturation? The results of this thesis indicate that there is a certain threshold to age, as photostimulation at wk 18 decreased egg production compared to photostimulation at wk 21 (Chapter 4), but also this question remained unanswered. There is hypothesized to be a delicate balance between photosensitivity and metabolic state, where an individual's intrinsic setpoints for body composition, BW, and photosensitivity, and their interactions, cannot yet be elucidated from external general parameters such as BW or time (age). Another question arising from this thesis is whether broiler breeders are ready to sexually mature when they are photosensitive or when physical development has met certain thresholds for lean, fat, or overall body mass, and, is there a certain balance between these characteristics? It was concluded in Chapter 4 that the rate of lay was lower for birds on the breeder recommended BW compared to birds with a higher BW, and rate of lay was lower for hens photostimulated at wk 18 compared to wk 21. In addition, in current lines of laying hens, feed intake capacity is too low for their level of production (Bouvarel and Nys, 2013; Pottguetter, 2015), i.e. birds remain in a negative energy, protein, and calcium balance. This indicates that even when birds commence egg production, their metabolic state, BW, or body composition may not be optimal for their full genetic potential of reproductive performance.

Optimal egg production efficiency in broiler breeders is only one tier in a sustainable broiler meat production chain. To investigate the transgenerational effects of the BW treatments described in Chapter 4 to 7 and the transgenerational effects of precision feeding, a research proposal was submitted by Dr Zuidhof and the author of this thesis which received funding as a Master thesis project. This Master thesis project was led and executed by Katelyn Humphreys and the results are in the process of publication. In summary, *ad libitum* fed male offspring from the High BW hens was 8.9% heavier and *ad libitum* female offspring was 7.4% heavier compared to male or female offspring from Standard BW hens at d 42. In addition, circulating levels of T4 were

11.7% higher in High BW offspring compared to Standard BW offspring. The maternal feeding system (Zuidhof, 2018) did not affect offspring performance. It is stressed that further research in this area is warranted to provide a complete picture for optimal broiler meat production.

In search for new evidence of the mechanisms linking metabolic status and reproductive performance in broiler breeders, certain components in study design need to be taken into consideration. One of the barriers of comparing studies investigating reproduction is that the onset of lay for the individually measured bird is not known or not reported and therefore the differences between treatments cannot be related to reproductive age or status, e.g. measurements cannot be adjusted for the age at sexual maturity (van der Klein et al., 2019; Chapter 6). Carryover effects of the rearing phase into the production phase should also be considered in studies investigating both phases. Breeds and line-specific details about stock (e.g. year) should also be noted, as the date of publication of results of academic studies does not always reflect the commercial strain available at the time when the research took place. Commercial broiler and layer breeds have significantly changed in the past and breeding companies continue to select their animals for new traits. When nutritional treatments are applied, it is important to record and present BW, BW change, feed allowance, feeding frequency, and feeding time, because outcomes of reproductive characteristics or endocrine levels may need to be adjusted for these factors, e.g. BW adjusted ovary weight or adjustment of pulsatile circulatory gonadotropin levels related to sampling time. In addition, if feeding treatments or feed treatment changes alone are subject of investigation, these should not coincide with other factors affecting (onset of) reproduction such as photostimulation; treatment effects can otherwise not be distinguished from other environmental cues. Differences in physiology and biology behind short term and long-term feed restriction might be substantial. It is suggested that new studies apply a more integrative approach where (neuroendocrine) signals from adipose tissue, hepatic tissue, and the gastrointestinal tract are investigated as well as their direct effect on both the ovary and the hypothalamus, such that local and system effects can be separated. Thus far, most studies investigating broiler breeders have focused on the effects of feed restriction or full feeding on the ovary and ovarian tissues and on systemic changes. More combined *in vitro* and *in vivo* work is needed to separate cause from consequence.

8.5 Implications for the industry

Precision feeding is not yet applied in the industry and, therefore, the results of this thesis are not directly translatable, as mentioned earlier in this thesis. However, some parts of this thesis can provide advice to the industry.

It can be concluded from this thesis that there is little value in estimating NE_g values for ingredients or feeds, as NE_g was affected by feed intake levels (Chapter 3). This emphasizes that non-feed sources of variation alter NE_g values. In addition, the heat increment of feeding did not depend on energy content of the diet. However, reaching sexual maturity reduced the heat increment of feeding (Chapter 7). The models described in both Chapter 3 and Chapter 7 can be used to better evaluate the fate of dietary energy. A better understanding of the partitioning of energy may lead to more precise formulation of diets and improved restrictions on least cost formulation. In addition, individually estimated RHP could be a potential characteristic for breeding selection indices, providing a tool for primary breeding companies to select for the most efficient birds.

For hatching egg producers, this thesis confirms the need for accurate and precise control of light schedules and stress the importance of light tight barns. However, suboptimal lighting programs were less problematic for heavier birds. Therefore, it is advised that producers find a balance in the timing of photostimulation that works for the BW and the variation in BW they experience in their flock. Ensuring birds in the lower end of the BW distribution are have reached a mature BW and sufficient adiposity will be required before applying photostimulation.

8.6 Conclusions

1. The non-linear model partitioning ME intake into maintenance and gain provided a non-invasive real-time method to measure HP and RE in broilers.
2. NE_g values of diets decreased when feed intake was reduced emphasizing that non-feed sources of variation altered NE_g values, an argument against implementing a NE system for poultry.
3. An interaction was shown between the effects of BW and rearing photoperiod on reproductive performance in broiler breeders. Greater BW or feed intake might override negative signals such as increased photoperiods against sexual maturation.
4. Although within-treatment variation in BW was minimized, decreasing the age at photostimulation from wk 21 to wk 18 increased variability in age at sexual maturity and decreased reproductive performance of broiler breeders.

5. The breeder-recommended BW at wk 21 is below the optimal target for sexual maturation after photostimulation for broiler breeders fed with a PF system.
6. The model describing E2 dynamics based on chronological age predicted that the duration between the E2 inflection point and age at first egg was longer in the birds fed toward the breeder recommended target compared to bird with a higher BW.
7. The model describing E2 dynamics based on physiological age predicted that the duration between photostimulation and the E2 inflection point was longer in birds fed toward the breeder recommended target compared to birds with a higher BW.
8. The modeling methodology for E2 dynamics revealed that the rate of E2 increase consistently peaked around 2.4 wk before sexual maturity, independent of BW or rearing photoschedule.
9. Including random parameters for both individual as well as age-related variation in ME_m requirements greatly reduced residual error and provided a biologically sound estimation of ME partitioning to maintenance, gain, and egg production for both the rearing and the laying phase.
10. In the laying phase, individual ME_m requirement was related to level of egg production and therefore level of feed intake.
11. Residual HP of hens on a breeder recommended target was lower compared to hens on a higher BW.
12. Reproductive status significantly affected the proportion of ME intake allocated to HP.
13. Heat increment of feeding was higher during the rearing phase compared to the laying phase.

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