Metabolic status and reproduction in precision-fed broiler breeders: Impact of growth trajectories

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

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Abstract

Broiler breeders are subjected to feed restriction programs to control excessive growth. However, current levels of feed restriction are becoming severe, raising welfare and suboptimal reproductive performance concerns in underfed breeders. To circumvent the issue, we studied new strategic growth curves in this thesis. Birds in all studies were fed using a precision feeding (**PF**) system.

The first study investigated the effect of minor feed restriction on feed efficiency in New Hampshire and Brown Leghorn strains. The growth data of the birds were fit to a mixed Gompertz model with bird-specific random coefficients for mature BW, rate of maturing, and both to evaluate the effects of random terms on the fitting and predictive performance of the models. The model with both random coefficients was determined to be the most parsimonious model. Minor feed restriction increased production efficiency, but this was not confirmed for the New Hampshire strain.

The second study was conducted with 40 broiler breeder pullets reared on one of ten target growth trajectories, which were designed with 2 levels of the amount of prepubertal growth (Standard g_1 and High g_1) and 5 levels of pubertal growth timing (I_2 that was advanced by 0, 5, 10, 15, or 20% of the coefficient estimated from the breeder-recommended target BW). Analysis of covariance showed that for every week of earlier I_2, 24 wk body fat increased by 0.38%; pullets came to lay earlier by 0.49 day; egg production increased by 0.33 egg/hen/d in the High g_1 treatment but decreased by 0.27 egg/hen/d in the Standard g_1 treatment, respectively. Increasing g_1 reduced feeding motivation index by 1.6 and 0.8 visits/meal during rearing and laying phase, respectively.

The third study investigated effects of the growth trajectories on energy efficiency of birds. It also tested the effects of chunking data into different sizes and inclusion of random terms associated with individual maintenance ME and ADG requirements on fitting and predictive performance of ME partitioning models. A model including a random term associated with individual maintenance requirement in a 3 wk chunk size was chosen as the most parsimonious based on greater fitting and predictive performance among the models. Standard g_1 treatment had lower residual heat production compared to the High g_1 treatment, indicating greater efficiency in utilizing the ME consumed.

The fourth experiment was an extension of the second experiment to evaluate the intergenerational effects of a reduced degree of maternal pre-pubertal phase growth restriction and earlier maternal pubertal phase growth on offspring growth and development. Two replicated broiler studies were conducted that varied in maternal age (35 and 42 wk). Overall, relaxed growth restriction during pre-pubertal and earlier pubertal growth increased male offspring growth by 2.2% and produced more efficient female broilers by reducing FCR by 0.017.

The fifth study evaluated some metabolic biomarkers that gave clues to the metabolic shifts resulting from sexual maturation. A total of 36 broiler breeder pullets were used, of which 30 were randomly assigned to one of 10 unique growth trajectories, and 6 were assigned to an unrestricted group. The growth trajectories varied in total gain in the prepubertal and pubertal growth phases ranging from the breeder-recommended target BW to 22.5% higher, in 2.5% increments. Increasing prepubertal and pubertal BW gains by more than 15% of the breeder-recommended target BW triggered fat metabolism and yolk precursor synthesis, which

consequently advanced sexual maturity. We concluded that certain metabolic signatures can be used to predict the metabolic status linked to the bird's maturity.

The sixth experiment was an extension of the fifth experiment to determine correlation between plasma concentrations of corticosterone measured by Enzyme Linked Immunosorbent Assay (**ELISA**) and Liquid Chromatography-tandem Mass Spectrometry (**LC-MS/MS**) methods. Plasma corticosterone levels were not affected by photostimulation BW, indicating the same welfare status between the precision fed high and low BW groups. Concentrations of plasma corticosterone measured using ELISA method were highly correlated ($r = 0.95$) with values measured using LC-MS/MS method.

In conclusion, the current breeder-recommended target BW is low for optimal reproductive performance. Increasing prepubertal BW gain by 10% and advancing the pubertal growth phase by 20% could increase margin over feed and chick cost for the hatching egg producers and the broiler chicken supply chain as a whole.

Preface

This thesis is an original work by Mohammad Afrouziyeh. Funding for the project described in Chapter 3 was provided by Alberta Agriculture and Forestry (Edmonton, AB), Egg Farmers of Canada (Ottawa, ON), and Egg Farmers of Alberta (Calgary, AB). For projects described in Chapters 4 to 8, funding from Alberta Agriculture and Forestry (Edmonton, AB), Poultry Innovation Partnership (Edmonton, AB), and in-kind support were provided by Xanantec Technologies Inc. and Aviagen.

Chapter 3 of this thesis has been published as "Improving a nonlinear Gompertz growth model using bird-specific random coefficients in two heritage chicken lines", and authored by Mohammad Afrouziyeh, René P. Kwakkel, and Martin J. Zuidhof. 2021. Poultry Science. 100:101059. ***https://doi.org/10.1016/j.psj.2021.101059***. I performed the trial, collected experimental data, conducted statistical analysis, and prepared the manuscript. I had a great opportunity to attend Wageningen University for a duration of 3 weeks where René P. Kwakkel provided guidance on statistical analysis especially about cross validation of the developed models in the study. Martin J. Zuidhof served as supervisory author and provided critical review of the manuscript. All co-authors read and approved the manuscript.

Chapter 4 of this thesis has been submitted to Poultry Science on January 27, 2021 and currently is under review. The manuscript has been submitted as "Timing of growth affected broiler breeder feeding motivation and reproductive traits", and authored by Mohammad Afrouziyeh, Nicole M. Zukiwsky, and Martin J. Zuidhof. I was responsible for the majority of data collection, statistical analyses and manuscript composition. Nicole M. Zukiwsky assisted with data collection. Martin J. Zuidhof assisted with statistical analysis and served as supervisory

author and provided critical review of the manuscript. All co-authors read and approved the manuscript.

Chapter 5 of this thesis has been submitted to Poultry Science on March 22, 2021 and currently is under review. The manuscript has been submitted as "Architecture of broiler breeder energy partitioning models", and authored by Mohammad Afrouziyeh, Nicole M. Zukiwsky, Jihao You, René P. Kwakkel, Douglas R. Korver, and Martin J. Zuidhof. I collected experimental data with the help of Nicole M. Zukiwsky and Jihao You. I managed and analysed data and drafted the manuscript. René P. Kwakkel, Douglas R. Korver, and Martin J. Zuidhof provided critical and constructive review of the manuscript. All co-authors read and approved the manuscript.

Chapter 6 of this thesis has been published as "Intergenerational effects of maternal growth strategies in broiler breeders", and authored by Mohammad Afrouziyeh, Nicole M. Zukiwsky, and Martin J. Zuidhof. 2021. Poultry Science 100:101090. ***https://doi.org/10.1016/j.psj.2021.101090***. I conducted two trials for this chapter. Nicole M. Zukiwsky assisted with data and sample collection in both trials. Martin J. Zuidhof served as supervisory author and provided critical review of the manuscript. All co-authors read and approved the manuscript.

Chapter 7 of this thesis has been submitted to Poultry Science on April 16, 2021 and currently is under review. The manuscript has been submitted as "Plasma metabolomic profiling reveals potential onset of lay biomarkers in broiler breeders", and authored by Mohammad Afrouziyeh, Nicole M. Zukiwsky, Douglas R. Korver, and Martin J. Zuidhof. I collected blood samples from the experimental birds with help from Nicole M. Zukiwsky. I participated in lab work which was done by The Metabolomics Innovation Centre, University of Alberta

(Edmonton, AB, Canada). Douglas R. Korver provided critical feedback on metabolism-related part of the manuscripts. I conducted statistical analysis on metabolomics data. Martin J. Zuidhof served as supervisory author and provided critical review of the manuscript. All co-authors read and approved the manuscript.

Chapter 8 of this thesis has been submitted to Poultry Science on May 14, 2021 and currently is under review. The manuscript has been submitted as "Comparison of liquid chromatography-tandem mass spectrometry and ELISA methods for measurement of plasma corticosterone in broiler breeders," and authored by Mohammad Afrouziyeh and Martin J. Zuidhof. I conducted all lab work on analysing blood samples using ELISA. I also participated in analysing the samples using LC-MS/MS method, which was done by The Metabolomics Innovation Centre, University of Alberta (Edmonton, AB, Canada). Martin J. Zuidhof served as supervisory author and provided critical review of the manuscript. All co-authors read and approved the manuscript.

Dedication

This thesis is dedicated to my mom's spirit, who brought me up and encouraged me to pursue my life goals wisely. Love you mom, even now that you are not in this world, I am sure you are in a better place.

Acknowledgements

Foremost, I would like to express my sincere gratitude to my advisor Prof. Martin J. Zuidhof for his patience, motivation, enthusiasm, and immense knowledge. Martin; I am so grateful for the skills you have taught me such as critical thinking skill and academic writing. You have been an incredible mentor who has been caring about his team. Your kindness is something that I will never forget!

I would like to thank my advisory committee members, Prof. René Kwakkel and Prof. Doug Korver. René; you are a pioneer in mathematical modelling science. I have learned a lot from you about modelling, especially during my stay in the Netherlands. You are also such a hospitable and modest person who always gives positive energy to his surroundings. I appreciate all your kindness! Dr. Korver; you were so generous to share your time with me and guide me throughout my Ph.D. program. It was also a great honour for me to serve your nutrition classes as a teaching assistant. It really helped me to enrich my knowledge.

I am gratefully thankful to my bride, Raheleh! My honey you are my dearest loved one forever. Thank you very much for your patience and help over the course of my Ph.D. program. You sacrificed a lot. I totally understand that and thankful for that. Love you and kiss you my bride!

I am also thankful to our elder son, Ali. Ali; you accompanied us with your great patience during our life journey. Love you so much, my beloved son! I am also thankful for our new-born baby, Ryan. Ryan; you are a special gift for us from God. Love you so much, my beloved son!

I want to thank all staff of the Poultry Research Centre, especially Shawn Rankin, who was a caring buddy. I never forget his kindness. I also gratefully thank technical assistance from Chris Ouellette, who was a real problem solver in technical difficulties. A special thanks to

Kerry Nadeau, Giles Hinse, Will Carney and Kim Thorsteinson for your technical support and guidance during my projects.

I would like to thank my colleagues and friends Nicole Zukiwsky, Jihao You, Katelyn Humphreys, Lauretta Ngere, Sheila Hadinia, Sasha van der Klein, and Thania Moraes for their support during my research.

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List of Abbreviations

ACF	Autocorrelation coefficient
ADFI	Average daily feed intake
ADG	Average daily gain
ADG_p	Positive average daily gain
ADG_n	Negative average daily gain
AL	ad libitum
AFE	Age at first egg
AIC	Akaike information criterion
ANOVA	Analysis of variance
AP	Alkaline phosphatase
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric pressure photo-ionisation
AUC	Area under the curve
b	rate of maturing
BHT	Butylated hydroxytoluene
BIC	Bayesian information criterion
BL	Brown Leghorn
BW	Body weight
BWFE	BW at first egg
BWPS	Body weight at photostimulation
Ca	Calcium
CON	Control
CORT	Corticosterone
CP	Crude protein
d	Day
DI/LC-MS/MS	Direct-injection liquid chromatography-tandem mass spectrometry
DW	Durbin Watson
E2	Estradiol
ELISA	Enzyme-linked immunosorbent assay
EM	Egg mass
ESI	Electrospray ionisation
EW	Egg weight
FCR	Feed conversion ratio
FSH	Follicle stimulating hormone
g_1	Total gain in the prepubertal phase
GnRH	Gonadotropin Releasing Hormone
h	Hour
H:L	Heterophil to lymphocyte ratio
HMW	High maternal weight
HPG	Hypothalamus-pituitary-gonad
HRP	Horseradish peroxidase
I_2	Pubertal growth phase inflection point
IGF-I	Insulin-like growth factor-I
IGF-II	Insulin-like growth factor-II

kg	Kilogram
LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
LED	Light emitting diode
LH	Luteinizing hormone
LSMeans	Least squares means
lx	Lux
m	Meter
MA	Maternal age
MAE	Mean absolute error
ME	Metabolizable energy
ME_m	Metabolizable energy for maintenance
MI	Maternal pubertal growth inflection
mL	Milliliter
MSE	Mean square error
MW	Maternal BW gain
P	Phosphorus
PLF	Precision livestock farming
PLS-DA	Partial Least Squares - Discriminant Analysis
SAS	Statistical analysis system
SEM	Standard error of the mean
SMW	Standard maternal weight
NH	New Hampshire
NLMM	Nonlinear mixed models
OPLS-DA	Orthogonal Partial Least-squares Discriminant Analysis
PF	Precision feeding
R^2	Coefficient of determination
ROC	Receiver operating characteristic
RFI	Residual feed intake
RFID	Radio frequency identification
RHP	Residual heat production
RMEm	Residual maintenance ME requirement
RMSE	Root mean square error
SD	Standard deviation
THP	Total heat production
VIP	Variable importance in the projection
wk	Week
Wm	Mature BW

1.0 Chapter 1. General Introduction

1.1 Introduction

The continuously growing market of broiler protein for the world population requires an increase in the number and efficiency of broiler breeders, the parent stock of meat-type chickens. Although there has been tremendous improvement in selecting broiler strains for high growth rate, feed efficiency, and breast meat yield traits for performance, reproductive efficiency of breeders has suffered due to a negative relationship between reproductive performance and growth rate (Renema and Robinson, 2004; Decuypere et al., 2010). Therefore, feed restriction is a standard practice in broiler breeder farms to control excessive growth. However, in contrast with increasing growth rate in broilers (Zuidhof et al., 2014), broiler breeder BW targets have changed very little over the past decades (Renema et al., 2007). Thus, the gap between growth potential of broilers and broiler breeder target BW is increasing, which has resulted in increased feed restriction intensity. Reducing feed consumption to the levels required to control BW has created welfare concerns in underfed breeders (van Krimpen and de Jong, 2014). Furthermore, some modern broiler breeder pullets do not have sufficient fat reserves to support egg production or even to undergo sexual maturation due to severe feed restriction (van Emous et al., 2015; van der Klein et al., 2018a; b; Zuidhof, 2018). Considering that the degree of feed restriction is adjusted based on a target growth curve, relaxing growth restriction, compared to the breeder-recommended target BW, needs to be investigated to alleviate the negative impact of severe feed restriction on broiler breeders. In addition, growth pattern and feeding regime can affect body composition (Sun et al., 2006) and metabolism of breeders (Renema et al., 2007; Hanlon et al., 2020), which subsequently can influence reproductive performance. It has been demonstrated that the growth performance of offspring can also be influenced by their parents' growth patterns

(van der Waaij et al., 2011; van Emous et al., 2015; Bowling et al., 2018). Therefore, it is prudent to investigate the intergenerational effects of maternal growth trajectories in broiler breeders.

Feed restriction is a method of feeding where feeding duration and amount of feed are limited; this definition is the primary type of feed restriction that has been considered in this thesis. Feed restriction programs have been used in meat-type chickens to control BW for robust reproductive fitness, increase production efficiency, and alleviate the incidence of some metabolic disorders (e.g., sudden death syndrome and ascites). Feed restriction programs can be designed strategically and systematically using mathematical models. Gompertz growth models have been used successfully to model longitudinal growth data in quail, partridges, and broiler chickens (Piao et al., 2004; Tholon et al., 2006; Sariyel et al., 2017; Tarôco et al., 2019; Weimer et al., 2020) as well as to design target growth trajectories (Zuidhof, 2020). In recent years, growth trajectories and concomitant feed restriction programs have been precisely implemented on individual animals using a precision feeding (**PF**) system (Zuidhof et al., 2017). The system uses radio frequency identification (**RFID**) to monitor real-time BW and feed intake data while allocating feed to individuals only when decided. In other words, sensor data collected in real-time through the PF technology allows for real-time management decisions, which contributes to precision livestock farming (**PLF**); the goal of PLF is to continuously monitor animals through a network of sensor technologies then compare animal performance to a standard, and make automatic adjustments (e.g. feed allocation) to optimize production (Werkheiser, 2018).

The current thesis has investigated feed restriction strategies in two heritage chicken lines from an efficiency standpoint and in broiler breeders from efficiency, welfare, metabolic status, reproductive performance, and intergenerational effects aspects using the PF system. More

specifically, the focus of this research was on using novel strategies to control broiler breeder BW and evaluate the effect of systematically and strategically relaxed growth restriction on their feeding motivation, reproductive performance, energy efficiency, metabolic status, welfare, and offspring performance.

Chapter 2 of this thesis discusses the literature to establish the state of knowledge in the broiler breeder growth management research area. It also introduces the application of mathematical models in PLF as inclusion of bird-specific random coefficients have been used to improve the traditional Gompertz growth models (Chapter 3), to design strategic and systematic growth trajectories for broiler breeders (Chapter 4), and to create novel nonlinear mixed-effect energy partitioning models (Chapter 5). More specifically, Chapter 3 elucidates the effect of including bird-specific random coefficients in a Gompertz growth model on the fitting and predictive performance of the model. Chapter 4 evaluates the effect of increased BW gain during prepubertal growth phase and earlier pubertal growth phase on hunger, reproductive performance, body frame size, and body fat in broiler breeder pullets and hens. Chapter 5 discusses the effect of growth trajectories on energy efficiency in broiler breeders. Interestingly, Chapter 5 also introduces a novel method in modeling the energy partitioning in broiler breeders using chunked data. More specifically, we divided BW and feed intake data into equivalent and elementary pieces of data before other data analysis steps, which is a novel method in preparing data for energy partitioning modeling purpose. Furthermore, the effect of including random terms associated with individual maintenance ME ($\mathbf{ME_m}$), ADG, and age in ME partitioning models on the fitting and predictive performance of the models were investigated. The effect of maternal growth trajectories (from Chapter 4) on offspring growth performance and body composition is discussed in Chapter 6. Broiler breeder plasma metabolome was analyzed at

various ages to identify biomarkers of sexual maturity and to provide a comprehensive understanding of the metabolome of broiler breeders during the pullet to hen transition period (Chapter 7). Chapter 8 determined the correlation between plasma concentrations of corticosterone (CORT) measured by ELISA and LC-MS/MS methods. Compared to ELISA, LC-MS/MS method is a faster assay with higher precision and reproducibility to measure steroid hormones, including CORT (Huan et al., 2014). Chapter 8 also investigated the effects of the high and low photostimulation BW and breeder age on plasma CORT levels. Finally, main contributions of the discussed projects for the industry, society, and science, the novelty of the projects, the limitations, the overall implications, and proposed future research are discussed in Chapter 9.

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2.0 Chapter 2. Literature Review

2.1 Defining the Problem

Broiler breeders, the parent stock of meat-type chickens, have high growth potential, which needs to be passed onto their broiler offspring. On the other hand, breeders need to produce fertile eggs, which requires an optimum body condition. Given a negative relationship between high growth rate and reproduction efficiency (Kerr et al., 2001; Decuypere et al., 2010; Siegel and Dunnington, 2017), broiler breeders are feed restricted to control excessive growth. Feed restriction is most severe during the rearing phase, where breeders receive about 45% of the feed allotment that a similar weight broiler receives (Widowski and Torrey, 2018). There is a growing body of evidence that the intensity of feed restriction has increased in broiler breeders over the past decades (van Emous et al., 2015; Renema et al., 2007). Severe feed restriction raises welfare concerns and causes suboptimal reproductive performance in broiler breeders (Riber et al., 2021; van der Klein et al., 2018a,b). The following literature review confirms that feed restriction has been increasing in broiler breeders. It discusses general and specific solutions to circumvent the issue of severe feed restriction and concludes that new growth strategies are needed for modern breeders to optimize breeder reproductive performance and welfare.

2.2 Broiler Breeder Management and Challenges

Broiler breeders, the parent stock of broilers, share the same genetic potential for fast growth and high feed intake as their progeny (Hocking et al., 1997; Bokkers and Koene, 2003; van Krimpen and de Jong, 2014). As far back as 1968, egg production, fertility, and hatchability of broiler breeder hens dropped due to over-production of large yellow follicles, which was defined as erratic oviposition and defective eggs syndrome (**EODES**; Jaap and Muir, 1968). The syndrome was characterized by multiple follicle hierarchies, non-rhythmic laying patterns, and

the high incidence of egg defects. Given that EODES could be controlled by restricting feed intake of breeders, it was hypothesized and confirmed that this syndrome was caused by *ad libitum* feeding of the broiler breeder hens (Hocking et al., 1987; Yu et al., 1992; Walzem et al., 1993). Similar problems, associated with *ad libitum*-fed broiler breeders, were reported a few decades later when breeder pullets had low peak lay, a decline in egg and chick production (Robinson et al., 1998; Katanbaf et al., 1989a,b), and high mortality rates around the onset of lay (Spradley et al., 2008). Eitan and Soller (2009) referred to these *ad libitum*-associated reproductive issues as "over-feeding complex" or "quasi-EODES condition. Therefore, the idea of restricting feed intake of broiler breeders was developed to control excessive BW gain during rearing, control ovary morphology, increase fertile egg and chick production, and livability during the laying phase (Robbins et al., 1986; Wilson and Harms, 1986; Hocking et al., 1987; Katanbaf et al., 1989a,b; Hocking, 1993; Renema and Robinson, 2004). Feed restriction is most severe in broiler breeders during the rearing period. The amount of feed allocated from 10 to 16 weeks of age is approximately one quarter of what *ad libitum*-fed individuals will eat (Savory et al., 1996; de Jong et al., 2002; Arrazola, 2019). There are different methods for feed restriction of broiler breeders, which will be discussed below.

2.2.1 Broiler Breeder Feed Restriction Programs

Broadly, feed restriction programs include quantitative and qualitative feed restriction methods. Quantitative feed restriction is managed either through a limited everyday feed restriction program or non-daily feeding schedules where birds receive no feed on one or more non-consecutive days per week and receive a greater quantity of feed on the remaining days. Non-daily feeding programs include skip-a-day, 4/3 (4 days fed and 3 non-consecutive fasting days) and 5/2 (5 days fed and 2 non-consecutive fasting days) feeding programs (Mench, 2002;

Carneiro et al., 2019). Non-daily feeding programs are limited to pullets. In limited everyday feed restriction, a restricted amount of feed is given to broiler breeders daily, whereas a skip-a-day program consists of feeding birds double the daily feed allocation every other day. de Beer et al. (2007) found that a skip-a-day feeding program was metabolically less efficient than everyday feeding in broiler breeders due to the need to deposit and remobilize nutrients during the fasting period. Zuidhof et al. (2015) reported that, conditioned to repeated energy shortages, skip-a-day fed birds compromised growth of breast muscle tissues and diverted more energy to storage in the abdominal fat pad. Skip-a-day feeding schedules are banned in some countries because of the welfare concern on not having a daily meal (DEFRA, 2007).

Qualitative feed restriction involves diluting caloric and nutrient content of diets typically by dietary soluble or insoluble fibers (Zuidhof et al., 1995; Savory et al., 1996; Tahamtani et al., 2020). This way, the amount of feed provided can be increased without increasing the total energy and nutrients intake (Sandilands et al., 2006). As a result, breeders may approach a higher level of satiety, as the gut content is increased compared to quantitative feed restriction (Hocking et al., 2004).

2.2.2 Concerns About Severe Feed Restriction in Broiler Breeders

Broiler growth potential has increased by 400% over a 50-year period. This selection for increased growth rate in broilers has led to an increase in adult BW in their parent stocks (Zuidhof et al., 2014). However, breeder-recommended broiler breeder BW targets have remained relatively constant over the past decades. More specifically, in 1979, the breeder target body weight was 53% of the broiler at 6 weeks of age, which reduced to 27% in 2005 and 22.5% in 2021 (Renema et al., 2007; Aviagen, 2019, 2021). This has created a considerable gap between growth potential of broilers and broiler breeder target BW. Reducing feed consumption

to the levels required to follow breeder-recommended target BW has increased the intensity of feed restriction. This leaves broiler breeders with an unrewarded foraging behaviour and likely also with a sensation of hunger and frustration, which can be associated with stereotypic object pecking, polydipsia (over drinking), and increased pacing (Riber et al., 2021). In addition, severe feed restriction has decreased body fat accumulation, which is necessary for the onset of sexual maturity. Thus, underfed modern breeder pullets are leaner compared to the classic ones and do not have enough body fat reserves to commence laying and sustain egg production (van Emous et al., 2015; van der Klein et al., 2018a,b; Zuidhof, 2018). As a result, modern broiler breeder management strategies need to evolve to alleviate the negative impact of severe feed restriction on reproductive performance and welfare.

2.2.3 Welfare in Broiler Breeders

In recent years, animal welfare has become an important aspect of livestock production and a focal point in scientific research (Sandøe et al., 2020; Häffelin et al., 2020; Giersberg et al., 2021). Although feed restriction is a common feeding strategy in broiler breeder management, it is associated with poor welfare (Mench, 2002, van Krimpen and de Jong, 2014; D'Eath et al., 2009; Tolkamp and D'Eath, 2016). Several parameters have been used as indicators of stress and welfare in broiler breeders. Feed-restricted broiler breeders may show signs of physiological stress in terms of high levels of plasma corticosterone (**CORT**; de Jong et al., 2003; Hocking et al., 1996; Mormède et al., 2007; Aranibar et al., 2020); cecal or colon digesta CORT (Post et al., 2003; Shini et al., 2008; Weimer et al., 2018), and feather content CORT (Bortolotti et al., 2008; Fairhurst et al., 2013; van Krimpen and de Jong, 2014; Carbajal et al., 2014). Hematological signs of stress include but are not limited to elevated blood heterophil: lymphocyte ratio, and low hematocrit (**HCT**) and packed cell volume (**PCV**; Scanes, 2016).

Several studies have shown that feed restricted broiler breeders show behaviour indicative of frustration (increase in activity level and decrease time spent for resting, eating, and comfort behaviour); more foraging behaviour; pacing; stereotypic object pecking; over drinking; and hyperactivity (Hocking et al., 2001; 2002; de Jong et al., 2003; Puterflam et al., 2006; Nielsen et al., 2011; Riber et al., 2021). It has been reported that aggressive pecking can be caused by factors such as hunger, feeding motivation and feeding frustration as a result of feed restriction (Mench 2002; Jones et al., 2004; Girard et al., 2017; Zukiwsky et al., 2021).

2.2.3.1 Corticosterone and Welfare

Classical studies on stress conducted by Hans Selye (1936) demonstrated that stress, as a syndrome, is characterized by physiological changes in the body. Stressors activate the hypothalamus-pituitary-adrenocortical (**HPA**) cascade, resulting in the release of CORT in birds (Blas, 2015; Palme, 2019). Plasma concentrations of CORT are elevated due to increased secretion of adrenocorticotropic hormone (**ACTH**) and corticotropin-releasing hormone. Circulating levels of CORT can increase in response to production stressors (e.g., heat, cold, stocking density, restraint, cooping, and shackling), and nutritional stressors including fasting, feed restriction, and dietary protein deficiency in chickens (Scanes, 2016). Yan et al. (2021) showed that feeding broiler chickens at 70% of *ad libitum* level elevated plasma CORT concentrations compared to that of *ad libitum*-fed birds. Feed restriction to the level recommended by the Hybro G broiler breeder guide increased plasma CORT concentrations compared to that of *ad libitum*-fed birds (de Jong et al., 2002). Feeding broiler breeder pullets at 75, 60, 45, and 30% of *ad libitum* feed intake increased plasma CORT linearly (Najafi et al., 2015). Increased CORT levels may be indicative of behavioral stress or be related to adaptive metabolic adjustments in the bird to cope with a decreased supply of energy. Aranibar et al.

(2020) compared the effect of a skip-a-day feeding program and an "alternative feeding program" on concentration of plasma CORT. The alternative feeding program, in their study, was feeding broiler breeders with soybean hulls on the off-feed day in a skip-a-day feeding program. Their results showed that plasma concentration of CORT was greater in both groups 48 h after consuming the feed compared to that of measured at 24 h after feeding. The authors concluded that the degree of feed restriction and the length of the fasting period between feedings had the most influence on plasma CORT level.

The following metabolic roles have been attributed to CORT: 1) Carbohydrate metabolism: CORT increases circulating concentration of glucose through greater rates of hepatic gluconeogenesis and reduced utilization of glucose in chickens (Li et al., 2009; Wang et al., 2012a; Zhao et al., 2012). 2) Lipid metabolism: CORT can increase fat accumulation in adipose tissues, liver, and skeletal muscle by increasing the synthesis of fatty acids (Cai et al., 2009; Wang et al., 2012a,b). However, increased lipolysis, as a result of glucocorticoid administration, has been shown in some studies, which was associated with elevated levels of non-esterified fatty acids (**NEFA**) in the blood. As such, CORT increased expression and levels of triglyceride lipase in chicken adipose tissue (Serr et al., 2011). 3) Protein metabolism: Decreases in protein synthesis and increases in degradation in skeletal muscle have been observed as CORT effects (Wang et al., 2015). Some effects of CORT on chicken metabolism are direct effects, but some are mediated by the increase in circulating concentrations of insulin (Cai et al., 2011; Song et al., 2011; Wang et al., 2012a). High levels of CORT can impair gut function by decreasing the expression of Na^{+}-dependent glucose transporter in jejunum brush border membrane vesicles (Li et al., 2009) and by increasing permeability of intestine membrane (Vicuña et al., 2015), which allows penetration of pathogens into the blood stream through the

intestinal barrier. CORT also plays an important role in energy metabolism. It supports the action of glucagon in mobilizing energy from the liver and adipose tissue (Kuo et al., 2015). An increase in the level of CORT in the post-absorptive phase is therefore a normal reaction in energy metabolism. Savory and Kostal (2006) indicated that the HPA axis has a metabolism-regulating function in the post-absorptive phase, which can increase heart rate, blood pressure, and body temperature in feed restricted birds. This is in line with Selye's concept of stress (Selye, 1936) in which CORT contributes to "fight or flight" responses through supplying an immediate energy source (e.g. blood glucose and NEFA). The authors concluded that these responses were more related to variations in feed intake and energy expenditure than to arousal or physiological stress.

2.2.3.2 Corticosterone Assays

In most studies, Enzyme Linked Immunosorbent Assay (**ELISA**) and Radioimmunoassay (**RIA**) have been routinely used for measuring CORT in chicken blood and feathers (Gonzales et al., 2003; Carbajal et al., 2014; Häffelin et al., 2020; Leishman et al., 2020; Cognuck et al., 2020; Weimer et al., 2020). A novel and faster assay to measure steroid hormones, including CORT, is liquid chromatography-tandem mass spectrometry (**LC-MS/MS;** Huan et al., 2014). It has been questioned whether the techniques employed have been adequately validated for chicken plasma or serum (Scanes, 2016). For instance, plasma concentrations of CORT in unstressed chickens vary between 0.3 and 20 ng/ml (Xie et al., 2015; Olanrewaju et al., 2014; Mirfendereski and Jahanian, 2015), whereas in stressed chickens, plasma CORT levels range from 0.25 ng/mL (Kang and Kuenzel, 2014) to ~150 ng/mL (Huang et al., 2014). Due to high variability (lack of precision) in plasma CORT levels obtained from colorimetric assays (e.g. ELISA and RIA

assays), validation of assays should be considered in research pertaining to CORT measures (Stanczyk et al., 2007; Zhou et al., 2011).

2.2.3.2.1 Enzyme Linked Immunosorbent Assay

The ELISA is a versatile and sensitive technique that is used for detection or quantification of practically any antigen, antibody, proteins, glycoproteins, and hormones in biological samples (Berzofsky et al.,1999). The sample containing the antigen of interest and an antibody with affinity for the antigen are incubated together to produce a measurable result (e.g. a color change). The intensity of the color change can be used to determine the amount of antigen in the sample. There are 4 types of ELISA (direct, indirect, sandwich, and competitive) that can be used to determine the concentration of a hormone (e.g. CORT) in a biological sample.

The direct ELISA begins with the coating of antigen of interest to the wells of ELISA plates. This process allows the operator to determine how much antigen is in the sample. In the next step, the plates are washed to remove any potential unbound antibody. Then any unbound sites on the ELISA plate are blocked using agents like ovalbumin, aprotinin, or other animal source proteins. The recent steps are important because they prevent the binding of any non-specific antibodies to the plate and minimize false-positive results. A primary antibody that is labeled with alkaline phosphatase (**AP**) or horseradish peroxidase (**HRP**) is then added to each well, which will bind specifically to the antigen of interest and result in a color change (Aydin, 2015). The color change occurs by either the hydrolysis of phosphate groups from the substrate by AP or by the oxidation of substrates by HRP. A strong color change indicates that there is a large amount of the primary antibody bound to the antigen. The degree of color change can be measured using a spectrophotometer. The intensity of the color change is used to quantify the

antigen concentration in the sample. Direct ELISA is the simplest form of ELISA. There is one antibody/antigen interaction in this ELISA type. Thus, there is little cross-reactivity in this technique. The pitfall of direct ELISA is the lack of sensitivity compared to other ELISA types (Engvall, 2010).

The indirect ELISA also requires coating of antigen of interest to the ELISA plates. This technique requires two antibodies, a primary detection antibody that attaches to the antigen of interest and a secondary enzyme-linked antibody complementary to the primary antibody. The primary antibody is added first, followed by a wash step, and then the enzyme-conjugated secondary antibody is added and incubated to produce a color change. The intensity of color change can then be measured using a spectrophotometer. The enzyme-labeled secondary antibody enhances the signal of the primary antibody. Thus, sensitivity of the indirect ELISA is greater compared to that of the direct ELISA. The greater sensitivity allows this assay to detect and quantify lower levels of antibody compared to the direct ELISA (Hnasko, 2015). The downfall of this method is that the enzyme-labeled secondary antibody can produce a high background signal, which can be cause by non-specific binding. The background signal reduces the accuracy of the ELISA; however, this issue can be controlled if the assay is conducted properly (Shah and Maghsoudlou, 2016).

The sandwich ELISA begins with a target-specific capture antibody coated onto the wells of the ELISA plates. The target-specific capture antibody has an affinity for the antigen of interest. The “sandwich” term is used because the antigens are sandwiched between two layers of antibodies (capture and detection antibodies). Once the sample is added to the wells, the antigen binds to the antigen specific capture antibodies. The detection antibody is then added that also has affinity for the antigen of interest. In the next step, a HRP conjugate is added, which binds to

the detector antibody and increases the strength of the color change. A 3,3',5,5'-Tetramethyl benzidine substrate is then added that reacts with the HRP to produce a color change. The sandwich ELISA has the highest sensitivity among all the ELISA types (Engvall, 2010).

A pre-coated well with a specific antibody is used for the competitive ELISA as well. The sample of interest is then mixed with a solution that contains tracer, or an enzyme-conjugated version of the hormone of interest. This mixture is then added to the wells of the ELISA plates where the antigen in the sample will compete for the binding sites with the enzyme conjugated version of the hormone. The competitive ELISA can measure a large range of antigens in a given sample. It also has low variability (Engvall, 2010; Aydin, 2015).

2.2.3.2.2 Liquid Chromatography-Tandem Mass Spectrometry

Liquid chromatography with tandem mass spectrometry is a robust analytical technique to detect and quantify a broad range of biological molecules. Mass spectrometry (MS) is a highly sensitive and accurate method in detecting the small biological compounds. Coupling of MS with the liquid chromatography (LC) method has provided some benefits in terms of the ability of the method to analyse complex mixtures with high specificity. Many compounds with a high degree of multiplexing can be measured in a single analytical run, which shows a fast-scanning speed in the LC-MS/MS technique (Pitt, 2009; Keevil, 2013).

To detect and quantify analytes of interest in a sample, the sample solution is pumped through a stationary phase (LC column) using a mobile phase flowing through at high pressure. The LC column separates the components of the sample based on the migration rate of each component through the column. The migration rate is affected by the chemical reaction between the components of the sample, the stationary phase, and the mobile phase. After elution from the LC column, the effluent is directed to the mass spectrometer. Two main steps in the mass

spectrometer are ionisation and ion analysis steps. In the ionisation step, the analyte molecules are converted to a charged state. There are several types of ionisation source in the mass spectrometer such as electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI) source, and atmospheric pressure photo-ionisation (APPI) source. Each of these ionisation sources has its own application. The ESI is the most common ionisation source for a wide range of biological molecules. However, there is limitation in efficiently ionisation of neutral and low-polarity molecules such as lipids by the ESI source. The APCI is a suitable ionisation source for small and thermally stable molecules that are not well ionised by the ESI source such as free steroids. Free steroids are neutral and relatively non-polar molecules without a functioning group capable of carrying charge. Therefore, the APCI source improves sensitivity of the LC-MS technique for quantifying the free steroids. Moreover, the APCI source is a reliable method for ionisation of lipids and fat-soluble vitamins. The APPI source is also suitable for ionisation of neutral compounds such as steroids. The APPI uses photons to ionise molecules after nebulisation, minimising concomitant ionisation of solvents and ion source gases. After ionisation step, the ions are analysed based on their mass per charge (m/z) ratio. This step scans across a range of m/z values, resulting in a mass spectrum. Product ion scans detect structural information about the analyte. In addition, they can detect identity of the analyte, that refers to a "finger-print" detection role of the scans (Pitt, 2009).

To quantify the analyte of interest concentration in a sample, a standard curve is used. To create a standard curve, internal standards are required. The internal standards are usually stable isotype versions of the analyte. These isotypes have the same chemical properties with those of the analyte and are easily detectable during the MS step. The standard curves plot analyte:

internal standard response ratio versus analyte concentration. Then, the standard curve can be used to quantify the analyte of interest in a sample (Kushnir et al., 2011; Pitt, 2009).

2.3 A Flashback to the Problem

Now that the severe feed restriction issues in broiler breeder management have been introduced according to literature, I would like to raise a broad question here to prepare the ground for introducing some solutions for this problem in later sections. The main broad question is: "What strategies have been investigated in literature to alleviate the negative consequences of severe feed restriction in broiler breeders?" To answer this, it is necessary to gain deep insight about some underlying mechanisms linking reproduction, welfare, and metabolic status of breeders; then, some available answers to the question will be provided according to the literature.

2.4 Requirements for Sexual Maturity in Broiler Breeders

2.4.1 Age, Body Weight, Body Conformation, and Body Composition

Some studies have shown that there is a minimum age and a minimum BW for the ability to respond to photostimulation and sexual maturation in broiler breeders (Katanbaf et al., 1989b; Lewis et al., 2007). Ciacciariello and Gous (2005) reported that age at sexual maturity in broiler breeder pullets can be advanced either by earlier photostimulation or by growing pullets at a faster rate to reach 2,100 g at 15 wk of age. Typically, recommended BW targets for broiler breeders (2,100 to 2,200 g) appear to be optimal for egg production (Lewis and Gous, 2006).

Leading up to the onset of lay, breeders should have optimum fleshing (body condition) with optimum levels of protein mass and fat tissue available. Skeletal frame size can be indirectly assessed by measuring shank length (Kwakkel et al., 1998). Robinson et al. (2007)

noted that feed restriction can limit shank length throughout the rearing period due to significant manipulation of the BW profile. There is evidence to suggest that a minimum amount of body fat may be required for broiler breeder pullets to reach sexual maturity (Bornstein et al., 1984; Sun et al., 2006). In broiler breeders, hens that had not entered lay prior to 55 wk of age had a fat pad which was 1.5% of their body weight, while those that had entered lay had a fat pad of 2.5%, suggesting that a minimum threshold does exist (van der Klein et al., 2018). It was shown that carcass fat at sexual maturity was between 11 and 15% of total BW (Joseph et al., 2000; Renema et al., 2001a;b; Sun et al., 2006; van Emous et al., 2015). Kwakkel et al. (1993) described the growth of the body and chemical components of laying hens in a multiphasic manner. The authors reported that after 11 wk of age, protein and fat deposition were mainly related to the development of the reproductive tract and abdominal fat deposition, respectively.

2.4.2 Mechanisms Linking Metabolic Status and Reproductive Axis in Broiler Breeders

To successfully stimulate sexual maturity and cause breeder pullets to come into persistent production with photostimulation, breeder pullets need to reach BW, body composition, and physiological thresholds within the context of hormonal balance. Major sites involved in attaining sexual maturity include Hypothalamus-Pituitary-Gonad (**HPG**) axis (maturation; Bédécarrats et al., 2016), ovary (through folliculogenesis and steroidogenesis; Mfoundou et al., 2021), liver (by formation of yolk lipids through lipogenesis; Wang et al., 2013), adipose tissue (through the effect of produced leptin and adiponectin on HPG axis; Hanlon et al., 2020), somatotroph (through the effects of growth hormone on insulin-like growth factor-I and the HPG axis; Hrabia, 2015), and thyroid axis (through modulation of effects of gonadotropins on ovarian function; Sechman, 2013).

The onset of sexual maturity involves the activation of the HPG axis. The secretion of gonadotropin-releasing hormone (**GnRH**) from the hypothalamus stimulates the release of the pituitary gonadotropins, luteinizing hormone (**LH**), and follicle stimulating hormone (**FSH**), which in turn activates gonadal development and release of sex steroids, including estradiol (**E2**) and testosterone. In female birds, E2 acts as a gonadotropin-inhibiting hormone (**GnIH**) inhibitor, thus indirectly promoting sexual maturation. GnIH acts to prevent the release of GnRH from the hypothalamus, and on the anterior pituitary to inhibit the synthesis and release of gonadotropins (Tsutsui et al., 2010). GnIH maintains the pullet in a juvenile state, and as maturation progresses, GnRH release dominates GnIH (Shimizu and Bédécarrats, 2010; Bédécarrats et al., 2009). In a sexually mature bird, high levels of E2 may also act as positive feedback to promote GnRH, and subsequent LH and FSH production (Bédécarrats et al., 2016). As seasonal breeders, chickens can utilize external environmental cues, mainly photoperiod, to initiate and terminate reproduction. There are deep brain photoreceptors located within the hypothalamus, which can respond to light signal as stimulus for activation of the HPG axis (Saldanha et al., 2001; Bédécarrats, 2015). Zukiwsky et al. (2021) investigated the effects of incremental increases in target BW gain, as well as non-restriction of broiler breeders, during prepubertal and pubertal growth phases on reproductive performance. The onset of lay depended on the degree of feed restriction, and some of the unrestricted pullets commenced egg production 2 wk prior to photostimulation. These results strongly suggest that modern breeders may rely on other cues, beyond photostimulation, to initiate reproduction. Therefore, it can be concluded that body composition, or metabolic status, or both have a role in triggering sexual maturation. Furthermore, it can be hypothesized that the metabolic status of the bird can stimulate activation of the HPG axis.

In avian species, the development of ovarian follicles is accompanied by the deposition of a large amount of yolk. In addition to having a role in follicle development, E2 stimulates liver production of egg yolk lipids and vitellogenin lipoprotein. In the liver, the formation of yolk-targeted very-low density lipoprotein (VLDLy) occurs. For this purpose, E2 stimulates the production of apoprotein on the VLDL surface that makes it invisible to the lipoprotein lipase enzyme, which would otherwise bind and breakdown the VLDL. Triacylglycerols (**TG**) are the main components of yolk lipid (Kuksis, 1992), which mainly are synthesized in the liver and secreted in the form of the VLDLy. When VLDLy and vitellogenin arrive at the yolk on the ovary, VLDLy remains intact, whereas vitellogenin is converted to end products called lipovitellin and phosvitin. Apolipoprotein B (apoB) and apolipoprotein VLDL-II (apoVLDL-II) are the VLDLy-associated apolipoproteins, and they are involved in the assembly of TG-rich lipoprotein particles. Avian apoB is a component of specialized VLDL particles that are produced by the liver in response to E2. ApoVLDL-II mRNA was specifically expressed in the liver and upregulated after laying (Yen et al., 2005; Wang et al., 2013).

Adipose tissue stores lipids and secretes a variety of hormones (adipokines) that influence physiological functions of organs related to growth, immunity, and reproduction. Adiponectin, an adipocytokine hormone, exclusively secreted from the adipose tissue, may take part in the initiation of preovulatory changes in the ovary and modulate the ovarian steroidogenesis process. At the hypothalamus level, adiponectin acts as a metabolic regulator of the reproductive functions via its influence on GnRH release in mice (Klenke et al., 2014). Maddineni et al. (2005) found that fasting in layer chickens decreased adiponectin mRNA quantity in the adipose tissue compared to an *ad libitum*-fed group. This could potentially impair initiation of sexual maturity.

The metabolic consequences of different feed restriction regimes have been studied in broiler breeders (Buyse et al., 2000; Kita et al., 2002; de Beer et al., 2007, 2008; Ekmay et al., 2010; Moradi et al., 2013). Nutritional status and the subsequent responses of key plasma metabolic hormones [insulin, glucagon, and triiodothyronine (**T3**)] are important factors that determine the level of hepatic lipogenesis in birds (Hillgartner et al., 1995), which is involved in formation of yolk lipids. Although the length of fasting period is different in various feed restriction regimes, fasting is known to influence many metabolic processes, shifting metabolism from anabolism to catabolism and from lipogenesis to lipolysis. In breeders, fasting reduces circulating T3, insulin, and insulin-like growth factor-I (**IGF-I**) levels (Sun et al., 2006; Moradi et al., 2013), whereas plasma glucocorticoid levels, insulin-like growth factor-II (**IGF-II**) and growth hormone were increased (Buyse et al., 2000; Kita et al., 2002). Likewise, feeding frequency can affect metabolic responses and reproductive efficiency; variations in nutrient intake and subsequent energy status are communicated to the liver and hypothalamic-pituitary axis by alterations in the plasma levels of hormones (e.g., insulin, glucagon, T3) and metabolites (e.g., glucose, free fatty acids). de Beer et al. (2007) found that skip-a-day feeding was less efficient than everyday feeding in breeders due to the need to deposit and remobilize nutrients during the fasting period. Shortening fasting length, through increasing feeding frequency, improved feed utilization efficiency that enhanced egg production rate and egg weight as well as reduced hepatic lipogenesis (Richards et al., 2003; Moradi et al., 2013).

The reproductive axis is vulnerable to the actions of hormones associated with the activation of the HPA axis (stress axis). In chickens, CORT impairs reproduction at the hypothalamic level by decreasing GnRH and increasing GnIH synthesis and release (Son et al., 2014), at the pituitary level by inhibiting LH secretion (Etches et al., 1984), and at the gonadal

level by inhibiting testosterone/estradiol release and ovarian function (Henriksen et al., 2011; Wang et al., 2013). There is evidence that intensive feed restriction can cause metabolic stress in terms of major fluctuation in energy balance, physiological stress responses, boredom, stereotypies, aggression, and other abnormal behaviors in poultry (Mench, 2002; van Krimpen and de Jong, 2014), which can contribute to increased physiological stress indices such as plasma CORT and dopamine (Najafi et al., 2015). Therefore, intensive degree of feed restriction may impair activation and function of reproductive axis in broiler breeders.

2.4.3 Energy Efficiency Indicators in Broiler Breeders

The classical energetic hierarchy defines metabolizable energy (**ME**) as the useable energy supplied to an animal from dietary nutrients, after accounting for faecal, gaseous, and urinary losses (Knox, 1979). ME intake lost as heat is equivalent to total heat production (**THP**) or the ME maintenance requirement (**ME_m**) of an animal (Zuidhof, 2019). The ME_m is expended for ingestion of feed, voluntary activity, immune response, and thermal regulation, which can be confounded by the individual variation and feed restriction level in broiler breeders (Zuidhof, 2019).

Feed conversion ratio (**FCR**) is a classic measure of feed efficiency in livestock animals. Feed conversion ratio has been calculated as input: output ratio (feed intake per unit of BW gain or egg production) in broilers (Skinner-Noble and Teeter, 2003; Lassiter et al., 2006), layers (Flock, 1998), and broiler breeders (de Beer and Coon, 2007). Feed conversion ratio does not account for variability in ME_m requirements; thus, FCR increases with age and BW because of higher ME_m requirements. Biological efficiency is estimated using feed as an energy input and BW gain, ME_m, and egg production as the main outputs in broiler breeders. Therefore, biological efficiency allows for adjustment of feed intake for all the expected uses of energy (BW gain,

ME_m, and egg production). Those animals that use less than the expected requirement have greater biological efficiency.

Residual feed intake (**RFI**) and residual heat production (**RHP**) are biological indicators of energetic efficiency of growth and egg production in poultry (Willems et al., 2013). Residual feed intake is defined as the difference between observed and predicted feed intake based on energy requirements for production and maintenance (Luiting, 1990; Kennedy et al. 1993). Residual feed intake is biased by differences in feed intake levels (Gabarrou et al., 1998) because in most cases, high-producing animals have higher feed intake, and extra feed intake increases heat increment of feeding. Although RFI accounts for ME_m requirements, it does not account for the heat increment of feeding (Swennen et al., 2007). Thus, RFI may not be the best indicator of the energetic efficiency of birds. Residual heat production or residual maintenance ME requirement (**RME_m**) is the residual of the linear relationship between ME_m and ME intake. Romero et al. (2009a) concluded that RME_m is not confounded by the 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. The authors indicated that hens with greater RME_m efficiency (lower ME_m) partitioned more energy toward chick production than those with low RME_m efficiency (higher ME_m). The slope of the linear equation between total HP (**THP**) and ME intake represents the proportion of increased ME intake that is lost as heat. It defines the linear rate of change of THP with respect to ME intake and is called heat increment of feeding. Estimated heat increment of feeding, as a percentage of the increase in ME intake, has been reported as 52% during the life-time of broiler breeders from 2 to 55 wk of age, 79% during the rearing phase from 2 to 20 wk of age, 44% during the laying phase from 22 to 55 wk of age (van der Klein et al., 2020a), 19 and 34% during the laying phase from 20 to 60 wk of age (Romero et

al., 2009a,b), and 87% from 10 to 23 wk of age (Hadinia et al., 2018). Animal factors such as age, composition of gain, and reproductive status (van der Klein et al., 2020a) and dietary factors such as diet composition (Romero et al., 2009a) can affect heat increment of feeding.

2.4.4 Evolution of Energy Partitioning Models in Broiler Breeders

Mathematical models have been developed to predict the fate of dietary energy in poultry. In such models, energy requirements are inferred by functions that minimize the variation between ME intake and its ultimate fates, which are primarily maintenance of the existing body and its tissues and functions, storage in the body (BW gain), and production of products (eggs). The final goal of developing these models is to understand the "unproductive" part of consumed energy (requirements for maintenance) and to reduce that part through managerial strategies such as targeted restricted feeding, or through breeding practices. Subsequently, reduction in maintenance requirements would increase the availability of energy for productive purposes such as growth and egg production. The basic energy partitioning model assumed constant energy requirements for maintenance (Byerly, 1941; Valencia et al., 1980; Byerly et al., 1980; Sakomura et al., 2003; Pishnamazi et al., 2008; Reyes et al., 2011, 2012). The classic form of this energy partitioning model was developed as follows:

$$MEI_d = a \times BW^{0.75} + c \times ADG + d \times EM + \varepsilon$$

where MEI_d = daily ME intake (kcal/d); a, c, and d = estimated coefficients; BW = BW (kg); ADG = gain (g/d); and EM = egg mass (g/d).

Romero et al. (2009b) let the exponent fluctuate to improve fit and reduce bias in energy partitioning models as follows:

$$MEI_d = a \times BW^{b} + c \times ADG + d \times EM + \varepsilon$$

Where the exponent of BW or scaling exponent (b) has previously been estimated as 0.54 (Romero et al., 2009a), 0.84 (Pishnamazi et al., 2015), 0.67 (Zuidhof et al., 2017), 0.68 (Hadinia et al., 2018), 0.51 (van der Klein et al., 2020a) for broiler breeders. Table 2.1 provides an overview of estimates for scaling exponent of BW along with experimental conditions in the above-mentioned studies. The variation in the estimation of the exponent (b) might be due to differences in age (pullet or mature birds), differences in feed allocation between studies, housing type, environmental conditions, or interactions between those factors. Romero et al. (2009b) further improved the energy partitioning model into a mixed-effect model, which allowed for estimation of maintenance requirements for individual birds. The authors included a normally distributed random term associated with the coefficient of metabolic BW by hen to separate individual variation linked to maintenance from other sources of random variation. They concluded that the mixed-effect nonlinear energy partitioning model had a lower Bayesian Information Criterion (**BIC**) compared to the fixed effect linear model, indicating a better fitting performance.

van der Klein et al. (2020a) developed 3 novel nonlinear mixed-effect models for broiler breeder lifetime: 1) by including random terms associated with the coefficient of metabolic BW to separate individual variation in ME_m from other sources of random variation; 2) by including a random term associated with the coefficient of metabolic BW by week to separate age variation in ME_m from other sources of random variation. 3) by including random terms for both individual maintenance and age where the age term was nested within the individual bird term. The authors concluded that including random terms for both individual and age in the energy partitioning model reduced residuals variation, indicating a robust model fitting performance. However, the authors did not evaluate predictive performance of the energy partitioning models.

2.4.5 Energy Requirements for Maintenance, Gain, and Egg Production in Broiler Breeders

Maintenance requirements take up a large portion of ME intake in chickens (Latshaw and Moritz, 2009). Maintenance ME is defined as the energy used to sustain an animal that is neither gaining nor losing weight in a post-absorptive state, in a thermoneutral environment, at rest, and in sexual repose (NRC, 1981). Estimates for ME_m requirement have ranged from 147.6 to 245.2 kcal/d for a 2.00 kg broiler breeder pullet or hen (Sakomura et al., 2003; Romero et al., 2011; Hadinia et al., 2018; van der Klein et al., 2020a). This wide range for ME_m requirement in the literature was due to differences in animal behavior, bird age, strain, temperature, different housing systems, feed intake level, and dietary energy level. For instance, breeders raised in floor pens had 20% greater ME_m requirement than cage-raised ones (Rabello et al., 2006). Sakomura et al. (2003) concluded that *ad libitum*-fed broiler breeder pullets had 10% greater heat increment compared to their feed restricted counterparts receiving 54% of the *ad libitum* feed intake. In addition, methodology being used to estimate ME_m requirement (indirect calorimetry, Spratt et al., 1990; comparative slaughter method, Rabello et al., 2006; Reyes et al., 2012; mathematical modelling approach, van der Klein et al., 2020b) could affect the estimated ME_m requirement. van der Klein et al. (2020b) compared mathematical modelling and comparative slaughter method in determination of heat production and concluded that the energy partitioning model [$MEI = (145.10 + u) \times BW^{0.83} + 1.09 \times BW^{-0.18} \times ADG^{1.19} + \varepsilon$] underestimated heat production by 13.4% compared with the comparative slaughter method. The authors acknowledged that the developed model in their study was not robust enough to accurately partition ME to heat production and retained energy. They suggested further studies to improve the model.

A wide range of ME requirements for gain has also been reported from 0.71 to 5.80 kcal/g in the literature (Sakomura, 2004; Reyes et al., 2012, Hadinia et al., 2018; van der Klein et al., 2020a). Composition of gain affects the ME requirements for growth. Several factors, such as stage of maturity and age affect composition of gain. Fat tissue contains a higher energy content (9.1 kcal/g) compared to lean tissue (5.5 kcal/g on a DM basis; Leeson and Summers, 2001). Energy requirement for fat deposition (10.9 kcal/g) is greater than that of protein deposition (8.63 kcal/g) in growing broilers. Moreover, as fat tissue contains less water compared to that of the lean tissue, the ME requirement per unit of fat tissue gain is much higher than those per unit of lean tissue gain (Lopez and Leeson, 2008). As age increases, the amount of body fat increases (Leenstra, 1986). Lean mass increases until egg peak production, and then net mobilization of lean tissue to support egg production causes a loss in lean tissue towards 50 wk of age in broiler breeders (Salas et al., 2010; van Emous et al., 2015; Vignale et al., 2016; Caldas et al., 2018). Caldas et al. (2018) reported the lowest point for lean tissue mass at 37 wk of age during egg production (from 23 to 59 wk of age) in broiler breeders. Egg production decreases after 50 wk of age; thus, the hen starts increasing lean tissue to prepare body composition for the next clutch or production cycle as happens in nature. However, fat accumulation occurs throughout the egg production phase and reaches a maximum at 50 wk of age (van Emous et al., 2015; Caldas et al., 2019). Thus, the ME requirement for gain increases as BW increases with age for energetically expensive fat mass at 50 wk of age.

The ME requirement for egg production ranges from 1.90 to 3.15 kcal/g (Sakomura, 2004; Romero et al., 2009b; Reyes et al., 2012; van der Klein et al., 2020a). The energy content of broiler breeder eggs ranges from 1.33 kcal/g (Sibbald, 1979) to 1.79 kcal/g (Chwalibog, 1992) with an average value of 1.54 kcal/g (Sakomura, 2004). With an average efficiency of ME

utilization for energy deposition in broiler breeder eggs (64%), an expected ME requirement for egg production would be around 2.40 kcal/g of egg (Sakomura, 2004). McLeod et al. (2014) created a model of ovarian follicle development, which is valuable for estimating energy requirements of laying hens. Approximately 84% of the retained energy in eggs is contained in the yolk. Gross energy partitioned for follicle development ($E_{retained}$) was a simple linear function of yolk volume ($E_{retained} = \Delta V \times \rho \times E_y$), where ΔV was the increase in the volume of the follicle (cm^3/d); ρ was the density of yolk (1 g/cm^3); and E_y was the energy content of whole yolk (3.01 kcal/g). Due to the high variability in the reported ME requirement for egg production in the literature, it is prudent to investigate some potential factors (e.g. length of periods in which production data are divided to) influencing the egg production requirements.

As discussed, there is variation in the reported broiler breeder ME requirements for by literature. Part of this variation might be related to inconsistent period lengths used to group BW and feed intake data (chunk size of experimental data) for modeling purposes. Dozza et al. (2013) employed an analytical method called data chunking, which divided data into equivalent, elementary pieces of data before other data analysis steps. Chunking data into different sizes was used to increase the robustness and sensitivity of parameter calculation by avoiding bias from data segments with heterogeneous durations. Although variety in data can be considered as an advantage in modeling, the variety caused by unexplained sources of variation can influence precision of the calculation of model coefficients leading to unreliability in using the models. The reported ME requirements in the literature were obtained using different chunk size of data, which could be a reason for the variability in the reported values. For example, ME requirement for egg production has been reported as 1.78 kcal/g in a semi-weekly chunked data (Pishnamazi et al., 2015); 2.10 kcal/g in a semi-weekly chunked data until 32 wk of age and weekly chunked

data thereafter (Romero et al., 2009b); 2.40 and 2.42 kcal/g in a weekly chunked data (Reyes et al., 2011; van der Klein et al., 2020). Therefore, the effect of chunking BW and production data to different sizes on the fitting and predictive performance of energy partitioning models remains to be elucidated.

2.5 Searching for Solutions

Let us come back to the previous question in this chapter: "What strategies have been proposed in the literature to alleviate the negative consequences of severe feed restriction in broiler breeders?" The strategies which have been proposed in the literature can be considered from a welfare perspective or a combination of welfare and production aspects.

Several strategies have been investigated to alleviate welfare problems associated with feed restriction, which includes multiple daily meals (de Jong et al., 2005); scatter feeding (Zuidhof et al., 2015; Tahamtani et al., 2020); diet dilution (Zuidhof et al., 1995; Savory and Lariviere, 2000); and using appetitive suppressants like calcium propionate (Morrissey et al., 2014; Sandilands et al., 2006). Tahamtani et al. (2020) investigated the effect of qualitative feed restriction on some indicators of welfare in broiler breeders at 19 wk of age. In their research, breeder diets were diluted using different types of fibres including oat hulls (insoluble fibre), oat hulls and sugar beet pulp (mixture of insoluble and soluble fibre), and maize silage (roughage). The authors concluded that although concentrations of plasma CORT did not differ among the treatments, assessment of fault bars (transparent bands in feathers produced under stressful and adverse conditions) showed lower stress in roughage group compared to other treatments. Similarly, Morrissey et al. (2014) concluded that broiler breeder pullets, raised under qualitative feed restriction (high fibrous diet with an appetite suppressant), showed good plumage condition,

suggesting low feather pecking due to reduced hunger sensation. This could have a positive effect on the skin condition, as intact plumage protects against skin injuries.

Relaxed feed restriction is a potential approach to reduce the intensity of feed restriction in broiler breeders (Hocking et al., 2002; Bruggeman et al., 2005; Zukiwsky et al., 2021). This approach alleviates both welfare and productivity concerns in underfed modern broiler breeders. Renema and Robinson (2004) reviewed consequences of severe feed restriction in broiler breeders, from both welfare and productivity perspectives. The authors highlighted the importance of redefining the normal or appropriate growth profile in broiler breeders as a solution to the dilemma of the appropriate feed management system. Hocking et al. (2002) found that increasing target BW by 20% at 18 wk of age did not affect egg or chick production. The authors reported no difference in welfare traits (measure of immune function, physiological indices of stress, and behavioral changes), which indicated no real benefit of the relaxed feed restriction protocols tested in their studies. Zukiwsky et al. (2021) increased broiler breeder target BW gain during prepubertal and pubertal phases incrementally up to 22.5% above the recommended BW target. Notably, the authors reported that relaxing growth restriction up to 22.5% above the recommended BW target decreased hunger in hens during the laying phase but not in pullets during the rearing phase. Zuidhof (2018) proposed two approaches to alleviate concerns about severe feed restriction and welfare in broiler breeders. The approaches include relaxing the degree of feed restriction or implementing new feeding technologies to ensure equitable feed distribution or a combination of both methods. This idea opens the window for using the concepts of precision livestock farming (**PLF**) in systematic evaluation of strategically designed growth curves in chickens.

2.6 Mathematical Growth Models and Precision Livestock Farming

Precision livestock farming is a form of livestock management with integrated technology, computers, and engineering (Wathes et al., 2008). The goal of PLF is to continuously monitor animals on a group or individual basis and their surrounding environment through a network of sensor technologies then compare animal performance to a standard, and make automatic adjustments (e.g. feed allowance) to optimize production (Werkheiser, 2018). Precision feeding is a category of PLF in which various feeding technologies are used to allocate the proper amount of feed according to specific nutrient requirements to an individual or a group of animals at the appropriate time (Pomar et al., 2014; Andretta et al., 2016). A precision feeding (**PF**) system was developed at the University of Alberta to feed every bird the right amount of the right feed at the right time (Zuidhof et al., 2019). It consists of smart feeding stations connected to a computer that communicates with every station and records accumulated data centrally. The PF system allows feed intake levels appropriate to achieve the target growth trajectories of each individual bird.

Mathematical models play an essential role in precision livestock farming as they help gain insight and understand a biological system's behavior by simplification, integration, and linkage of parts. Mathematical models themselves may not be the primary objective of a study but they are part of a broader data-analysis approach to problem solving. Models can be used to explain observed patterns and to develop tools for predictive and decision-making purposes (Haag and Kaupenjohann, 2001).

Understanding animal growth is important for optimized management and feeding practices as well as genetic improvement of animals. For example, estimating age-specific BW provides a valuable basis for estimation of energy requirements, and thereafter feed intake

(Emmans, 1987). Growth in animals in general is a very complex phenomenon influenced by genotype as well as by environmental factors including nutrition. Growth in nature is a sigmoidal-shape phenomenon which can be separated into several superimposed phases (pre-pubertal, pubertal, and post-pubertal) based on the allometric growth of the body parts (Zuidhof, 2020). Mathematical models have been used to describe growth curves and account for the sigmoid, asymptotic nature of animal growth. Modelling the growth curves of animals is particularly important for optimizing the management and efficiency of animal production. More specifically, growth models describing genotype-specific growth curves can be used to visualize growth patterns over time and generate equations that can predict the expected BW of animals at specific age; suitable slaughter age; and age of sexual maturity. In fact, growth models help us estimate the daily energy, protein, and mineral dietary requirements (Norris et al., 2007; Darmani-Kuhi et al., 2011, Nahashon et al., 2010, Kaplan and Gürcan, 2018; Do and Miar, 2020). These equations result in mathematical growth parameters that are biologically interpretable (Tzeng and Becker, 1981; Aggrey, 2002; Zuidhof, 2020). For example, for feed-restricted animals, the biologically meaningful growth parameters would facilitate the design and study of alternative growth strategies.

2.6.1 Multiphasic Gompertz Growth Model

Several growth equations have been used to fit growth data of animals, among them non-linear mathematical models, such as Gompertz, Brody, Von Bertalanffy, Logistic, and Richards have been quite widely used to describe growth curves (Topal et al., 2004; Aggrey, 2009; Karaman et al., 2013; Schinckel et al., 2005; Wurzinger et al., 2005; Kohn et al., 2007). Narinç et al. (2017) reviewed different growth curve analyses in poultry science and concluded that the Gompertz growth model was the most commonly used growth model in the literature since 1970.

The authors indicated that the Gompertz model was the best-fitting model in growth curve analysis of meat-type chickens, quail, and turkey. Moreover, best-fitting model for a quail line with decreased weight was Logistic and for a line with increased weight was Gompertz. Yakupoglu and Atil (2001) used Gompertz and Von Bertalanffy models to analyse weekly BW of Cobb and Hubbard commercial broiler flocks and recommended the Gompertz growth model as the most robust model. The Gompertz growth function (Gompertz, 1825) or modifications thereof (Tjørve and Tjørve, 2017) have been used to describe the BW-age relationship. With improvement of computational tools in the past years, multiphasic models, and multiphasic mixed-effect regression models have been proposed to estimate individual variation in growth parameters (Kwakkel et al. 1993; van der Klein et al. 2020a; Zuidhof, 2020). Mixed-effect regression models are quite robust to various violations from modelling assumptions such as homogeneity of variance and lack of autocorrelation among data (Aggrey, 2009; Gibbons et al., 2010). In addition, mixed-effect models account for individual variation in growth rates and mature body sizes, which we know exist in populations (Wang and Zuidhof, 2004). Thus, not only treatment effects, but variation in model coefficients due to unique individual differences, such as an individual animal's specific and unique genome, its environment, and its stage of life, can also be estimated.

2.6.2 Designing Strategic and Systematic Growth Trajectories

In the previous sections, the value and biological relevance of growth models were explained. Growth curves can be designed strategically using robust mathematical models. This can be done through derivation and manipulation of model parameters including asymptotic weight, rate of attainment of mature weight, and the age at which an animal attained the inflection point of the curve (Barbato, 1991). Continuous growth parameters include total

amount of gain accruing in each growth phase, rate of growth in each phase, and the inflection point of each growth phase or age at which growth for each phase reaches its maximum rate. Multiphasic Gompertz models can be developed to implement a robust hypothesis-based approach for optimization of growth curves (Zuidhof, 2020). Continuous growth parameters in the models can be altered strategically to formulate hypotheses related to growth trajectories. The resulting growth trajectories can subsequently be implemented and evaluated in a systematic way. For instance, we can decrease rate of growth for the early growth phase and subsequently increase it for a later growth phase to design compensatory growth programs for broilers. Another example would be relaxing the early growth restriction during the rearing phase for severely feed-restricted modern broiler breeders to let the body accumulate enough body reserves (e.g., fat) for sexual maturation (Zuidhof, 2020).

2.7 Heritage Chickens

As I used heritage chickens in research to develop a novel mixed-effect Gompertz growth model, the importance of these chickens is explained in this section. Over the past century, massive replacement of low-productivity local breeds with high productivity ones has reduced genetic resources, which has raised concerns about animal biodiversity (Caballero et al., 2010; Cendron et al., 2021). Therefore, using heritage genotypes in research projects could help to increase poultry biodiversity given that purebreds are reared on a limited number of farms and little knowledge is available on their performance (Rizzi et al., 2013). Heritage chickens are important for breeders and industry to protect valuable genes and traits over the long term. However, 50% or more of the genetic diversity in ancestral breeds is absent in commercial pure lines (FAO, 2007; Muir et al., 2008). Therefore, some institutions promoted several recovery programs by conducting research with heritage breeds to preserve their genetic diversity (Zanetti

et al., 2011). Preserving heritage breeds allows conserving the traits of adaptability, required in future environmental and production conditions, to promote animal adaptation. Hence, heritage breeds could be considered as robust components for crossbreeding to generate more resistant and adaptable commercial lines (Soglia et al., 2020).

New Hampshire, Brown Leghorn, White Leghorn, Light Sussex, Plymouth Rock, and Rhode Island Red are examples of heritage chickens being conserved at the University of Alberta in Canada. The New Hampshire breed originated from the state of New Hampshire in the United States. At the Poultry Research Centre of University of Alberta, the New Hampshire has the following characteristics: bred as a dual-purpose bird (egg and meat production); fast and early rate of maturity; calm and curious; early feathering; brown egg producers; average mid-cycle egg weight is 53 g; average female weight is 2,100 g; and the average male weight is 2,650 g. Brown Leghorns originated in Italy and were introduced to America in 1853. They have the following characteristics: very active; early growth rate with medium rate of maturity; and noted for hardiness and vigour (Nassar et al., 2012; Lambertz et al., 2018; Fulton et al., 2016). We used New Hampshire and Brown Leghorn breeds in the current thesis.

2.8 Intergenerational Effects of Maternal Growth Patterns in Broiler Breeders

Maternal nutrition affects broiler BW, carcass composition (van der Waaij et al., 2011; van Emous et al., 2015; Bowling et al., 2018), and skeletal and muscle development (Saccone an Puri, 2010). Maternal growth can also affect broiler offspring growth performance and body composition. Increasing target BW and the amount of feed available to broiler breeders increased offspring hatch BW (van der Waaij et al., 2011) and final BW (van der Waaij et al., 2011; van Emous et al., 2015; Bowling et al., 2018). Maternal feed restriction intensity can affect offspring abdominal fat deposition. van der Waaij et al. (2011) found that offspring of feed-restricted

breeders had significantly lower BW and relatively more abdominal fat deposition compared to those of breeders fed *ad libitum*. They concluded that it might be due to a mismatch between maternal and offspring feeding levels and nutritional environment, which would potentially lead to economic loss and impaired feed efficiency. Maternal growth and nutrition affect offspring through epigenetic effects or altering nutrient composition in the egg during embryo development.

Epigenetics is defined as heritable changes in gene function without change in DNA sequence that can influence phenotype (Scholtz et al., 2014). Immune function, behavior, temperature regulation, and response to stress and growth efficiency in chickens can be altered by epigenetic mechanisms, and all these factors can change the rate of DNA methylation or histone modification of certain sections of DNA (Li et al., 1993; Bélteky et al., 2018; Kisliouk et al., 2017). Moraes et al. (2019) examined the effects of diluting maternal dietary energy (2,800 vs. 2,900 kcal/kg of diet) and CP level (15.3% and 13.7%) during the broiler breeder rearing and laying phases on progeny growth performance. The authors concluded that the low maternal ME level reduced growth performance of male offspring broilers. Changes in energy:protein ratios require a metabolic adjustment by the animal, which they hypothesized may have triggered an epigenetic effect and influenced gene expression relating to growth and breast muscle development in the offspring.

Previous studies showed that increasing maternal target BW by 8% at 20 wk of age (Fattori et al., 1991), 20% at 18 wk of age (Hocking et al., 2002), 8% at 20 wk of age (van Emous et al., 2013), and 16 and 20% at 20 wk of age (Gous and Cherry, 2004; Ekmay et al., 2012) did not affect average egg weight. However, in other research implementing higher target BW by 21% (Renema et al., 2001a,b) and 13% (Sun and Coon, 2005) at 20 wk of age increased

egg weight. It has been reported that egg size is an important factor in chick weight, chick quality, and performance of broiler chicks to market weight (Abiola et al., 2008; Iqbal et al., 2016; 2017), whereas others have found that any advantage of chicks hatched from large-sized eggs diminishes rapidly after hatching (Pinchasov, 1991; Yannakopoulos and Tserveni-Gousi, 1987).

Several research studies have indicated that male and female offspring responded differently to maternal nutrition, which may be related to epigenetic sex-specific genes that affect body composition in the offspring (Spratt and Leeson, 1987; van Emous et al., 2015; van der Waaij et al., 2011). Bowling et al. (2018) found that increasing dam BW by 15% increased male broiler BW by 8.5% compared to the standard dam group. The authors further found that the concentration of yolk CORT of low BW hens was 1.2 times that of high BW hens and suggested that male embryos may be more sensitive to maternal feed restriction-induced stress. Humphreys (2020) reported that broilers from high BW hens (21% above the recommended BW) were 3.9 and 4.1% heavier than broilers from standard BW hens on day 35 and 42, respectively. This research also found that gut and abdominal fat pad weights were 6.4 and 16.0% greater in broilers from high BW hens compared with standard BW hens, respectively. Therefore, it is necessary to investigate the intergenerational effects of maternal growth trajectories on broiler growth performance and body composition. It would also be of great value to analyze the economic impacts of maternal growth treatments on the supply chain as a whole.

2.9 Preparing for Tomorrow, Today

To decrease the gap between broiler breeders and their offspring target BW and to circumvent adverse production effects of severe feed restriction on welfare and productivity of breeders, it is necessary to define relaxed growth restriction patterns during the prepubertal and

pubertal growth phases. In such a way, broiler breeders would have sufficient body reserves to commence sexual maturity and sustain a productive laying cycle. With the improvement of computational tools in the past years, multiphasic growth models and multiphasic random regression models have been proposed to estimate growth parameters. These growth parameters can be used to design novel growth trajectories systematically. In a common sense, the fitting and predictive performance of the classic growth models need to be evaluated and improved. In this way, it is cost-effective to use heritage chickens to conduct research on improving the mathematical growth models on one hand and preserve the local genetics on the other hand. The newly developed PF system at the University of Alberta facilitates precise implementation of various growth trajectories on chickens, controlling feed intake and monitoring BW and feed intake of free-run chickens. The PF stations allow one bird to eat at a time, without interference from other birds. This allows us to control the level of feed intake of each bird. In addition, using robust mixed-effect energy partitioning models to track the consumed energy fate allows studying the energy efficiency in birds raised under different growth patterns. Moreover, further research is needed to elucidate mechanisms linking metabolic status and the reproductive axis in broiler breeders. This will provide a better understanding of the physiological mechanisms driving the interaction among growth trajectory, feed allocation, sexual maturity, metabolic status, and reproductive axis. Given that plasma concentration of CORT is used as an indicator of animal welfare, it is necessary to validate the CORT assays including ELISA and LC-MS/MS methods. More specifically, the results of a colorimetric enzyme reaction (e.g. ELISA) can be confounded by many environmental factors, which could decrease precision of the assay. However, LC-MS/MS method produces more reproducible results. Furthermore, investigating the effects of maternal growth patterns downstream in the broiler supply chain will provide

insight on intergenerational effects of growth trajectories. This information can be used to optimize growth trajectories in broiler breeders.

2.10 Objectives

The objectives of the current thesis were as follows:

1. To evaluate the fitting and predictive performance of the classic Gompertz growth model and improve it, by explaining previously unexplained sources of variation, through inclusion of bird-specific random effects using two heritage chicken lines (Chapter 3).
2. To investigate the effect of minor feed restriction on production efficiency of two heritage chicken lines (Chapter 3).
3. To evaluate the effect of increased BW gain during prepubertal growth phase and earlier pubertal growth phase on hunger, reproductive performance, body frame size, and body fat in broiler breeder pullets and hens (Chapter 4).
4. To investigate the effect of relaxing intensity of feed restriction in prepubertal growth phase and implementing earlier pubertal growth on energy efficiency of broiler breeders (Chapter 5).
5. To evaluate inclusion of random terms associated with individual ME_m, ADG, and age in a ME partitioning model on model fitting and predictive performance and residual dependency (Chapter 5).
6. To evaluate how including random terms associated with individual maintenance ME, ADG, and age could bias and improve the ME partitioning model (Chapter 5).

7. To evaluate the effect of dividing BW, ADG, and egg production data into different chunk sizes (daily, 4-d, weekly, 2-wk, or 3-wk) on fitting and predictive performance of ME partitioning model (Chapter 5).
8. To investigate the effect of a reduced degree of maternal pre-pubertal phase growth restriction and earlier maternal pubertal phase growth on offspring growth and development (Chapter 6).
9. To understand differences in metabolism during pre- and post-pubertal phases by evaluating the effect of lay status (pullet vs. hen), photostimulation BW, and onset of lay timing (early vs. late) on plasma metabolomic dynamics (Chapter 7).
10. To determine correlation between plasma concentrations of CORT measured by ELISA and LC-MS/MS methods (Chapter 8).
11. To investigate the effects of the high and low photostimulation BW and breeder age on plasma CORT levels (Chapter 8).

2.11 Hypotheses

It was hypothesized that:

1. Minor feed restriction (feeding 95% of the ad-lib counterpart) would reduce RFI, thereby increasing production efficiency (Chapter 3).
2. Inclusion of bird-specific random coefficients in the classic Gompertz growth model would increase fitting and predictive performance of the model (Chapter 3).

3. Increasing BW gain during prepubertal growth phase and earlier pubertal growth phase would decrease hunger and increase body frame size, body fat, and reproductive performance in broiler breeder pullets and hens (Chapter 4).
4. Increasing BW gain during prepubertal growth phase and earlier pubertal growth phase would reduce biological energy efficiency (residual feed intake) in broiler breeders compared with their counterparts raised based on the breeder-recommended target growth (Chapter 5).
5. Increasing data chunk size in developing mixed-effect energy partitioning models would reduce unexplained variation in data, increasing the fitting and predictive performance of the models (Chapter 5).
6. Increasing maternal pre-pubertal phase growth and earlier maternal pubertal growth phase would increase offspring hatch BW, final BW, and digestive tract weight (Chapter 6).
7. Lower maternal BW in broiler breeders would increase fat pad weight in their broiler offspring (Chapter 6).
8. Lay status (pullet vs. hen) and onset of lay timing (early vs. late) would affect plasma metabolome, which can be used as a tool to understand the correlations between metabolic status and reproductive status in broiler breeders (Chapter 7).
9. Increasing photostimulation BW would decrease circulating CORT concentration in broiler breeder plasma (Chapter 8).
10. There would be high correlation between plasma concentrations of CORT measured by ELISA and LC-MS/MS methods (Chapter 8).

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Table 2. 1. Overview of estimates for scaling exponent of BW (b in BW^b) in energy partitioning models[1] in Ross 708 broiler breeders.

Scaling exponent of BW	Bird age	Housing system	Temperature (°C)	Feed allocation	Reference
0.54	Mature	Individual	21	Based on two target BW curves (10% greater or 10% lower than standard BW target)	Romero et al., 2009a
0.84	Mature	Individual	15, 19, 23, and 27	Identical feed allocations within diet (Low ME; 2,790 kcal/kg and High ME; 2,912 kcal/kg)	Pishnamazi et al., 2015
0.67	Pullet	group	Not reported	Based on two treatments (CON: standard target BW curve interpolated hourly and STEP: standard BW curve updated every 21 d)	Zuidhof et al., 2017
0.68	Pullet	group	20.8 ± 0.34	Feed allocation was based on weekly BW recording in a skip-a-day feeding program and based on real-time BW data in a precision fed treatment to maintain breeder recommended target BW	Hadinia et al., 2018
0.51	Lifetime	group	20.7	Based on two target BW curves (to reach the standard target BW curve or to an accelerated target BW curve reaching the 21-wk BW at wk 18)	van der Klein et al., 2020a

[1]Main form of an energy partitioning model is $MEI_d = a \times BW^b + c \times ADG + d \times EM + \varepsilon$ (Romero et al., 2009b); where MEI_d = daily ME intake (kcal/d); b = scaling exponent of BW; a, c, and d = estimated coefficients; BW = BW (kg); ADG = gain (g/d); and EM = egg mass (g/d).

3.0 Chapter 3: Improving a Nonlinear Gompertz Growth Model Using Bird-Specific Random Coefficients in Two Heritage Chicken Lines

3.1 Abstract

Growth models describe BW changes over time, allowing information from longitudinal measurements to be combined into a few parameters with biological interpretation. Nonlinear mixed models (NLMM) allow for the inclusion of random factors. Random factors can account for a relatively large subset of the total variance explained by bird-specific measurement correlation. The aim of this study was to evaluate different NLMM using birds from two heritage chicken lines; New Hampshire (NH) and Brown Leghorn (BL). A total of 32 birds (16 mixed sex birds from each strain) were raised to 17 wk of age. After 12 wk, half were continued on ad libitum (AL) feed intake, and half were pair-fed, using a precision feeding system; they were given 95% of the AL intake of a paired bird closest in BW. Residual feed intake (RFI) of birds, as an indicator of production efficiency, was increased in pair-fed BL birds as a result of minor feed restriction. Growth data of the birds were fit to a mixed Gompertz model with a variety of different bird-specific random coefficients. The model had the form: $BW = Wm \times exp^{-exp^{-b(t-t_{inf})}}$; where Wm was the mature BW, b was the rate of maturing, t was age (d), t_{inf} was the inflection point (d). This fixed-effects model was compared with NLMM using model evaluation criteria to evaluate relative model suitability. Random coefficients, $Wm_u \sim N(0,V_{Wm})$ and $b_u \sim N(0,V_b)$, were tested separately and together and their differences, for strains, sex and feeding treatments, were reported as different where $P \leq 0.05$. The model with both random coefficients was determined to be the most parsimonious model, based on an assessment of serial correlation of the residuals. NLMM coefficients allow stochastic prediction of the mean age and its variation that birds need to achieve

a certain BW, allowing for unique new decision support modeling applications; these could be used in stochastic modeling to evaluate the economic impact of management decisions.

Key words: Gompertz, growth model, heritage, multiple random coefficient, nonlinear mixed model

3.2 Introduction

Heritage chickens are important for breeders and industry to protect valuable genes and traits over the long term. However, 50% or more of the genetic diversity is absent in commercial pure lines (FAO, 2007; Muir et al., 2008). Therefore, preserving potentially valuable genes by conducting research with heritage breeds is important. Changes in live weight and proportional growth of body components as affected by genotype and environmental factors are defined as growth, while those changes appearing in growth over time are defined as growth curves (Camdeviren and Tasdelen, 2002). Growth curves are widely used for mathematical descriptions of growth in which growth parameters can be interpreted in a biological context (Kahm et al., 2010; Narinc et al., 2014a). Growth curves can be used to describe genetic potential of growth, estimating daily nutrient requirements for different ages and genetic groups, improving efficiency of livestock production, detecting a measurable growth trait, getting information about the health status of farm animals, determining the most suitable slaughtering age, and evaluating the effect of selection programs on the parameters of a growth curve (Lopez et al., 2000; Narinc et al., 2014b; Schinckel et al., 2005). Analysing and interpretation of growth parameters should be carefully considered because precise growth models can help develop strategies to ensure that animal production is efficient and cost-effective. Growth models describing genotype-specific growth curves can be used to dynamically estimate daily nutrient requirements at different ages, resulting in matching nutrient supply to the nutrient requirement. Robust ability to predict the

growth pattern of individual birds is necessary to optimize poultry production systems. The Gompertz growth function describes a general sigmoidal growth curve and has been used for fitting the BW data of different animal species with a large range in body size (chickens: Aggrey, 2002; pigs: Schinckel and Craig, 2002; and dairy cattle: Perotto et al., 1992). The Gompertz growth function is usually estimated once per genotype (multiple animals), such that a common mature weight (Wm) is estimated for each genotype, and a random error (eit) is associated with each individual bird i at age t in the model, which is assumed to be independent and normally distributed with mean of zero and a constant variance σ2. Since there is no random effect associated with Wit (weight of bird i at age t) in this model, it is a fixed effect model. The outcome of such models would be imprecise as they would not account for individual variation in growth rates and mature body sizes, which we know exist in populations (Wang and Zuidhof, 2004).

Growth data usually consist of repeated measurements over time on multiple subjects. Although longitudinal data provide more information than cross-sectional data, some challenges, such as heterogeneity of variance and correlated errors of measurement, are associated with their analysis (Gibbons et al., 2010). For instance, heavier birds are typically heavier at multiple adjacent measurement points over time, and this will increase heterogeneity and auto-correlation issues in growth data. Mixed-effects regression models, which are widely used for analysing longitudinal data, are quite robust to the various violations from modelling assumptions such as homogeneity of variance and lack of auto-correlation among data. Furthermore, in contrast to traditional regression techniques, mixed-effects models are able to estimate fixed and random parameters simultaneously which result in more accurate estimation for fixed parameters and their standard errors (Jiang and Li, 2010). Although several nonlinear mixed models (NLMM)

have been used to model growth data (Aggrey, 2009; Karaman et al., 2013; Schinckel et al., 2005), the effect of accounting for individual sources of variation in growth models on the estimation accuracy of growth parameters has not been fully investigated, and to our knowledge are mostly new to the poultry science literature.

Efficiency of production is increasingly important with escalation of feed costs and demands to minimize the environmental footprint. In this regard, improved growth models as well as precision feeding (PF) would offer an opportunity to match nutrient supply to nutrient requirements of individual birds; this would result in improved production efficiency (Zuidhof, 2020). Residual feed intake (RFI) is a biological indicator of energetic efficiency and defined as the difference between observed and predicted feed intake based on energy requirements for production and maintenance (Luiting, 1990; Kennedy et al. 1993). It has been reported that feed restriction increased production efficiency by lowering RFI (Metzler-Zebeli et al. 2019). Therefore, we hypothesized that minor feed restriction (feeding at the 95% of the ad-lib counterpart) would reduce RFI, thereby increasing production efficiency.

The objectives of the current study were 1) to evaluate different nonlinear mixed models with and without inclusion of random coefficients to account for knowable individual sources of variation using birds from two heritage chicken lines 2) to obtain estimated values for random coefficients of growth parameters including growth rate and mature BW; 3) to investigate the effect of minor feed restriction on production efficiency.

3.3 Materials and Methods

The animal protocol for the study was approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP00000121) and followed the Canadian Council on Animal Care guidelines and policies (CCAC, 2009).

3.3.1 Study Design

The current experiment consisted of a 2 × 2 × 2 factorial arrangement of treatments, with 2 heritage strains, New Hampshire (**NH**) and Brown Leghorn (**BL**), 2 sexes (male and female), and 2 feeding levels (ad libitum (**AL**) and restricted in which they were given 95% of the AL intake of a paired bird closest in BW). Each bird was an experimental unit.

3.3.2 Birds, Housing, and Management

A total of 32 birds (16 mixed-sex birds from each strain) were kept in an environmentally controlled facility at a stocking rate of 6.0 birds per m^2 from hatch to 17 weeks of age. The birds were housed in a single pen containing 4 precision feeding stations. All birds on both treatments were fed individually by a PF system (Zuidhof et al., 2017) that could apply the feeding treatments to each individual bird. Therefore, every bird was an experimental unit. Room temperature was maintained at 33°C during the first 2 d, and from d 3 onwards temperature was gradually reduced to 20°C by wk 5. A commercial standard mash starter diet was provided from 1 to 28 d of age, followed by a mash developer diet from 28 to 119 d of age. The ME (kcal/kg), CP and digestible Lys were 2,800, 19.00 and 1.00% for the starter, and 2,980, 16.45 and 0.75% for the developer, respectively. Water was provided AL throughout the experiment. At 25 d of age each individual bird was equipped with a wing band containing a radio frequency identification (RFID) transponder to be recognized by the PF system. Body weight and feed intake data were recorded by the PF system for each individual bird throughout the experiment. Pair feeding was done from 12 to 17 wk of age. At 12 wk of age, feed restricted birds were paired with a bird closest in BW (23 ± 14.6 g difference in BW of two pair birds), and pair-fed at 95% of the AL intake of its match. Pair feeding was implemented using the PF system software. Therefore, PF system was able to

identify the birds' RFID and then provide the right amount of feed based on the treatment of the bird.

3.3.3. The Nonlinear Mixed Effect Gompertz Model

Four Gompertz functions were evaluated. The following fixed effects model was the basic model:

$$BW = Wm \times exp^{-exp^{-b(t-t_{inf})}} [1]$$

where Wm was mature BW; b was rate of maturing; t was age (d) and t_{inf} was inflection point (d).

Models with inclusion of random coefficients, either Wm_u or b_u for individual Wm and b respectively, were considered in models 2 and 3:

$$BW = (Wm + Wm_u) \times exp^{-exp^{-b(t-t_{inf})}} [2]$$

$$Wm_u \sim N(0, V_{Wm})$$

$$BW = Wm \times exp^{-exp^{-(b+b_u)(t-t_{inf})}} [3]$$

$$b_u \sim N(0, V_b)$$

Finally, the model with inclusion of both random coefficients (Wm_u and b_u) is shown in model 4:

$$BW = (Wm + Wm_u) \times exp^{-exp^{-(b+b_u)(t-t_{inf})}} [4]$$

$$Wm_u \sim N(0, V_{Wm})$$

$$b_u \sim N(0, V_b)$$

The estimated fixed-effect parameters were Wm, b and t_{inf}; these were population-level estimates of mature BW, rate of maturing, and inflection point, respectively. The random-effect

parameters were Wm_u and b_u, and these accounted for bird-specific variation in mature BW and rate of maturing, respectively.

3.3.4 Calculation of Residual Feed Intake

Observed energy intake was calculated by multiplying the observed daily feed intake (g) by the dietary energy content (kcal/g). Predicted energy intake was estimated using an empirical energy intake model and accounted for energy used for maintenance and BW gain. A nonlinear model of ME intake as a function of metabolic BW and average daily gain (ADG) was used to estimate RFI which is shown in model 5 (Romero et al., 2009).

$$MEI = a \times BW^b + c \times ADG \times BW^d + \varepsilon \quad [5]$$

where MEI was ME intake (kcal/d); a was estimated maintenance requirement or the average total heat production from 8 to 17 weeks of age (kcal/kg^b); BW was body weight (kg); c (kcal/g) was the coefficient representing energy requirement for gain (ADG, g/d); b and d were exponents for BW to calculate the degree to which BW affected the energy cost of maintenance and gain, respectively; ε was residual or unexplained error.

Then RFI was calculated as

$$RFI = \text{observed MEI} - \text{predicted MEI}$$

3.3.5 Statistical Analysis

BW measurements were fit to the fixed effects model [1] and random effects models [2, 3 and 4] using the NLMIXED procedure in SAS (Version 9.4, SAS Institute Inc., Cary, NC, 2012). The NLMIXED procedure was used to fit the energy partitioning model (equation [5]) as well. The energy initial values of covariance parameters for running PROC NLMIXED were obtained from covariance matrix of individual parameter estimates. Under normality assumptions, minimized value of −2 log-likelihood as well as the Akaike information criterion (**AIC**), Bayesian

information criterion (**BIC**), and corrected version of AIC (**AICC**), provided by the software, were used for the evaluation of alternative models in terms of their fitting performance (Akaike, 1974; Schwartz, 1978). The criteria were computed as follows:

$$AIC = 2f(\hat{\theta}) + 2p$$

$$AICC = 2f(\hat{\theta}) + \frac{2pn}{n - p - 1}$$

$$BIC = 2f(\hat{\theta}) + p\log(s)$$

where f was the negative of the marginal log-likelihood function, $\hat{\theta}$ the vector of parameter estimates, p was the number of parameters, n is the number of observations, and s is the number of subjects. Lower values of these statistics reward preferred goodness of fit of the model to the data among alternative models, rewarding a more accurate explanation of variance. Adding more parameters into the model penalizes the fit statistic. For example, AIC will increase by 2 for every additional parameter (p) estimated. Growth parameters and their relevant random coefficients were estimated using the Dual Quasi-Newton optimization technique (Al-Baali and Fletcher, 1985). For models containing random effects, the Adaptive Gaussian Quadrature method was used as an integration method. A K-fold cross validation method was used to evaluate the predictive performance of the models. The dataset was randomly partitioned into 5 (K = 5) mutually exclusive equal subsets using the SURVEYSELECT procedure of SAS, and the procedure was repeated 10 times. Each time, K-1 subsets were used as a training set and one subset was used for testing. The R-square of the relationship between observed and predicted BW; the mean absolute error (**MAE**); the mean square error (**MSE**); and the root mean square error (**RMSE**) were calculated as cross validation statistics for the testing data (Yang and Huang, 2014). Cross validation statistics were computed as follows:

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|yi - \hat{y}i|$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}i)^2$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}i)^2}$$

where y_i was the i^{th} BW observation, $\hat{y}i$ was the predicted value for the i^{th} BW observation, and n was the number of observations.

For non-linear mixed models, SAS provides no straightforward way to assess the serial correlation of the residuals. To get insight into the residual serial correlation, we plotted the lag residuals versus residuals for each model and conducted a regression analysis to compare R-square and regression coefficients of the models (Gooijer and MacNeill, 1999). Three-way analyses of variance were conducted on growth random coefficients (Wm_u and b_u) using the MIXED procedure of SAS, with the feed allocation treatment, sex and strain as sources of variation. RFI was analyzed as a 4-way ANOVA using MIXED procedure in SAS where feed allocation treatment, sex, strain, and period (pre-pair-feeding and pair-feeding) considered as source of variations. Pairwise differences between means were determined with the PDIFF option of the LSMEANS statement and were reported as different when $P \leq 0.05$. Trends were reported where $0.05 < P \leq 0.10$.

3.4 Results

3.4.1 Model Comparison

Figure 3.1 illustrates the residuals for fixed-effects and random-effects models. Inclusion of random effects accounted for bird-specific variation in Wm and b resulted in reduced bias (systemic error) in prediction of individual BW through increasing the homogeneity of residual variation. This confirms that the random effects accounted for a considerable amount of variation in the dependent variable. Estimates of Nonlinear Gompertz growth parameters obtained with fixed and mixed effects models along with model selection criteria are presented in Table 3.1. The residual variance decreased for models [2], [3] and [4] as compared to model [1] by 90.7, 96.4 and 98.1%, respectively which indicated that more variation was accounted for. By incorporating a random effect into the fixed effects model, part of the error variation (σ^2_e) was partitioned into bird-specific variation in Wm (σ^2_{Wm} in model [2]) and b (σ^2_b in model [3]), resulting in a lower residual variance for the models [2] and [3]. Further decline in the residual variance of model [4] was a result of further partitioning the error variation into individual differences in mature BW (Wm), the rate of maturing (b), and their covariance ($\sigma_{Wm,b}$). The fitting criteria infer that model [4] was the preferred model because it diverted appropriate bird-specific variation from the residual error term. The log-likelihood, Akaike and Bayesian information criteria were all lower in model [4] compared with other models. Cross validation statistics summarizing the predictive performance of the models is presented in Table 3.1. The coefficient of determination (R^2) for random-effects models [2, 3, and 4] was higher than that for the fixed-effect model [1]. Model [4] had the highest coefficient of determination ($R^2 = 0.996$) indicating that 99.6% of the variation in predicted BW of the testing data was explained by the observed BW data. Furthermore, model [4]

had the lowest MAE, MSE, and RMSE among the models. Therefore, for the current dataset, model [4] should be considered the model of choice to predict growth.

Table 3.2 shows regression analysis of lag residual against residual for all models used in this trial to assess the serial correlation of the residuals. The coefficient of determination (R^2) for random-effects models [2, 3, and 4] was lower than that for the fixed-effect model [1], with the lowest one for model [4] (R^2=0.51) which means that only 51% of the variation in lag-residual in model [4] was explained by the residual. In other words, it showed a low degree of the relationship between adjacent residual and residual which was preferred. Therefore, it could be concluded that including both random effects of mature BW (Wm_u) and rate of maturing (b_u) in model [4] reduced auto-correlation bias in longitudinal growth data.

3.4.2 Estimated Growth Parameters

Table 3.3 shows the estimated mature BW and rate of maturing along with their relevant random coefficients for model [4]. Overall, Wm and b estimates for both the fixed and random effects models were similar (Wm = 2.00 ± 0.106 and b = 0.0273 ± 0.00046) because the expected means of the mixed effect model were the same as that of the fixed effect model and the data were balanced for all birds. The values for random coefficients of the growth parameters were bird-specific and should be interpreted such that Wm for the BL strain was 0.299 kg less than the overall mean Wm (2.00 kg), or 2.00 – 0.299 = 1.701 kg; correspondingly, Wm for the NH strain was 2.00 + 0.468 = 2.468 kg, and so on for all effects tested. Within the NH strain, the random coefficient for mature BW (Wm_u) was greater for males, and for the AL treatment, and within the BL strain, Wm was greater for males and the AL treatment.

3.4.3 Production Efficiency

RFI data, as a sense of production efficiency, are presented in Table 3.4. An interaction effect was seen among feeding level, strain, and period (P = 0.008; Figure 3.2). The RFI was decreased with age for all groups, i.e. the birds became more efficient as age advanced (post-pair-feeding period compared to the pre-pair-feeding period). RFI of the AL NH birds decreased in the second period, while there was not same decrease in RFI for feed restricted group during the second period. However, minor feed restriction decreased RFI in BL strain, indicating an increased efficiency. The interaction effect among sex, strain, and period (P = 0.042) indicated greater efficiency for female BL and male NH during the second period as age advanced. There was a trend to reduced RFI for restricted-fed males compared to AL males (P = 0.055).

3.5 Discussion

The pair-feeding results indicated a sex and strain-dependent effect of a minor feed restriction on production efficiency. In this regard, the results of other research (Mebratie et al. 2017) showed a clear sex by genotype interaction in broilers which indicated that the male BW records had a considerably larger residual environmental variance than female records, which means female records are more informative than male records. Overall, minor feed restriction increased production efficiency, but this was not confirmed for NH strain in the current study.

In this study, a nonlinear mixed-effects growth model was developed for growth data of NH and BL birds. The growth model with two random parameters for Wm and b was found to be the most parsimonious model based on fit statistics, and further analysis showed that it reduced auto-correlation bias in longitudinal growth data. The mixed-effects model provided an estimation of random coefficients for growth parameters of different subsets of the population. Mature BW (Wm) and rate of maturing (b) could be used in genetic selection programs. These random

coefficients could be used as a tool in different scenarios of poultry production system such as stochastic prediction of BW of individuals at any age to better match nutrient supply to nutrient requirements, and to predict and evaluate the economic impact of management decisions on designing target growth curves, breeding programs, and nutritional management decisions.

3. 6 Acknowledgment

Funding from Alberta Agriculture and Forestry (Edmonton, AB), Egg Farmers of Canada (Ottawa, ON), and Egg Farmers of Alberta (Calgary, AB), the Poultry Research Centre (Edmonton, AB), and technical assistance from Chris Ouellette and Mehdi Heidari is gratefully acknowledged.

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3.8 Tables

Table 3. 1. Estimated growth parameters, standard errors, and model selection criteria from fixed- and mixed-effects growth models for mixed-sex New Hampshire and Brown Leghorn heritage chickens.

Model [1]	Model [1]			Model [2]			Model [3]			Model [4]		
Parameter [2]	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|
Wm	1.98	0.0505	< 0.001	1.97	0.097	< 0.001	2.94	0.029	< 0.001	1.99	0.105	< 0.001
b	0.027	0.0009	< 0.001	0.027	0.0001	< 0.001	0.017	0.0006	< 0.001	0.027	0.0004	< 0.001
σ^2_e	0.053	0.0013	< 0.001	0.0019	4.8E-5	< 0.001	0.0049	0.0001	< 0.001	0.001	2.5E-5	< 0.001
σ^2_{Wm}	-	-	-	0.2985	0.075	0.004	-	-	-	0.34	0.087	0.004
σ^2_b	-	-	-	-	-	-	1.2E-5	4.6E-6	0.013	5.8E-6	2.34E-6	0.10
σ_{Wmb}	-	-	-	-	-	-	-	-	-	-0.00042	0.00029	0.15
Model fitting statistics [3]												
-2log-likhood	-263			-10,733			-7,714			-12,679		
AIC	-255			-10,723			-7,704			-12,665		
AICC	-255			-10,723			-7,704			-12,665		
BIC	-230.7			-10,716			-7,697			-12,655		
Cross validation statistics [4]												
MAE	0.1565			0.0304			0.0565			0.0183		
MSE	0.0548			0.0019			0.0049			0.0010		
RMSE	0.2342			0.0439			0.0705			0.0317		

R-square	0.824	0.993	0.984	0.996

[1] Model [1]: Fixed-effects model $BW = Wm \times exp^{-exp^{-b(t-t_{inf})}}$
Model [2]: Mixed-effect model including a random effect (Wm_u) for Wm
Model [3]: Mixed-effect model including a random effect (b_u) for b
Model [4]: Mixed-effects model including random effects (Wm_u and b_u) for both Wm and b.
[2] Wm= mature BW; b= rate of maturing; σ^2_e= residual BW variance; σ^2_{Wm}= the individual variance in mature BW; σ^2_b= the individual variance in rate of maturing; σ_{Wmb}= individual covariance between random effects
[3] AIC = Akaike information criterion; BIC = Bayesian information criterion
[4] MAE = Mean absolute error; MSE = Mean square error; RMSE = Root mean square error; R-square = Coefficient of determination of observed BW with predicted BW by the testing model in a k-fold cross validation.

Table 3. 2. Regression analysis for lag-residual versus residual for fixed- and random-effect growth models for mixed-sex New Hampshire and Brown Leghorn heritage chickens.

Model [1]	Model [1]			Model [2]			Model [3]			Model [4]		
Parameter	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|
Intercept	0.00038	0.0004	0.35	0.0003	0.0004	0.35	0.0005	0.0004	0.19	0.0004	0.003	0.29
Slope	0.97	0.0017	< 0.001	0.84	0.0091	< 0.001	0.91	0.0058	< 0.001	0.71	0.012	< 0.001
R-square		0.98			0.72			0.88			0.51	

[1] Model [1]: Fixed-effects model $BW = Wm \times exp^{-exp^{-b(t-t_{inf})}}$; Model [2]: Mixed-effect model including a random effect (Wm_u) for mature BW (Wm); Model [3]: Mixed-effect model including a random effect (b_u) for rate of maturing (b); Model [4]: Mixed-effects model including random effects (Wm_u and b_u) for both mature BW (Wm) and rate of maturing (b).

Table 3. 3. Estimated mature BW + random coefficient (Wm + Wm_u) and rate of maturing + random coefficient (b + b_u) for strain, sex and treatments by growth model [4] including random effects of mature BW and rate of maturing for mixed-sex New Hampshire and Brown Leghorn heritage birds under ad libitum and restricted feed intake pair-fed treatments.

		Average deviation of random coefficient from the population average					
		Strain [2]		Sex		Treatment [3]	
Parameter [1]	Population average	BL	NH	Female	Male	AL	Res
Overall Wm (kg)	2.00						
Wm_u		- 0.299	0.468	- 0.324	0.493	0.246	- 0.077
SEM	0.106	0.0490	0.0462	0.0413	0.0533	0.0455	0.0497
P-value		< 0.001		< 0.001		< 0.001	
Overall b	0.0273						
b_u		- 0.00034	0.00028	0.00028	- 0.00035	- 0.00117	0.0011
SEM	0.00046	0.00062	0.00058	0.00052	0.00067	0.00057	0.00063
P-value		0.48		0.47		0.014	

[1] Wm: mature BW (Wm); Wm_u: random coefficient for Wm; b: rate of maturing; b_u: random coefficient for b.

[2] BL: Brown Leghorn; NH: New Hampshire

[3] AL: Ad libitum; Res: Feeding level; AL: Ad libitum; Res: Restricted from 12-17 wk of age at 95% of the AL intake of a paired bird closest in BW

Table 3. 4. Residual feed intake of mixed-sex New Hampshire and Brown Leghorn heritage chickens under ad libitum and restricted feed intake pair-fed treatments prior to and during pair feeding

Effect		RFI (kcal/d) [1]	SEM
Period			
	Pre-pair-feeding	17.200	2.833
	Pair-feeding	-4.878	3.609
FL [2]			
	AL	12.660	4.050
	Res	-0.338	4.445
Sex			
	Male	13.564	4.758
	Female	-1.241	3.678
Strain [3]			
	BL	-0.728	4.367
	NH	13.050	4.134
P-value			
	Period	<0.0001	
	FL	0.045	
	Sex	0.025	
	Strain	0.035	
	FL × Sex	0.055	
	FL × strain	0.372	
	Strain × Sex	0.380	
	FL × Period	0.447	
	Strain × Period	0.951	
	Sex × Period	0.765	
	FL × Strain × Sex	0.390	

FL × Strain × Period	0.008
FL × Sex × Period	0.812
Sex × Strain × Period	0.042
FL × Sex × Strain × Period	0.839

[1] RFI: Residual feed intake

[2] FL: Feeding level; AL: Ad libitum; Res: Restricted from 12-17 wk of age at 95% of the AL intake of a paired bird closest in BW

[3] BL: Brown Leghorn; NH: New Hampshire

3.9 Figures

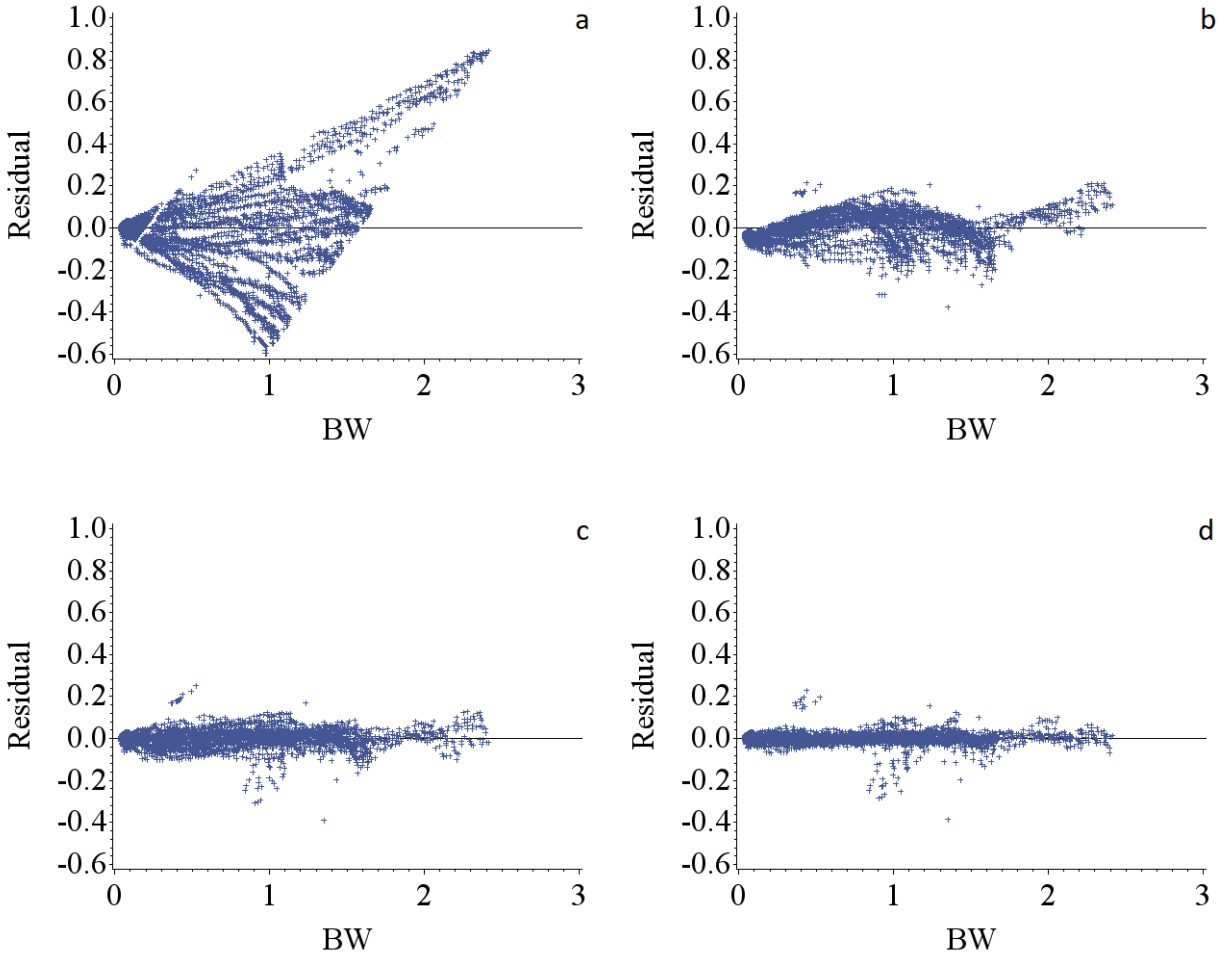
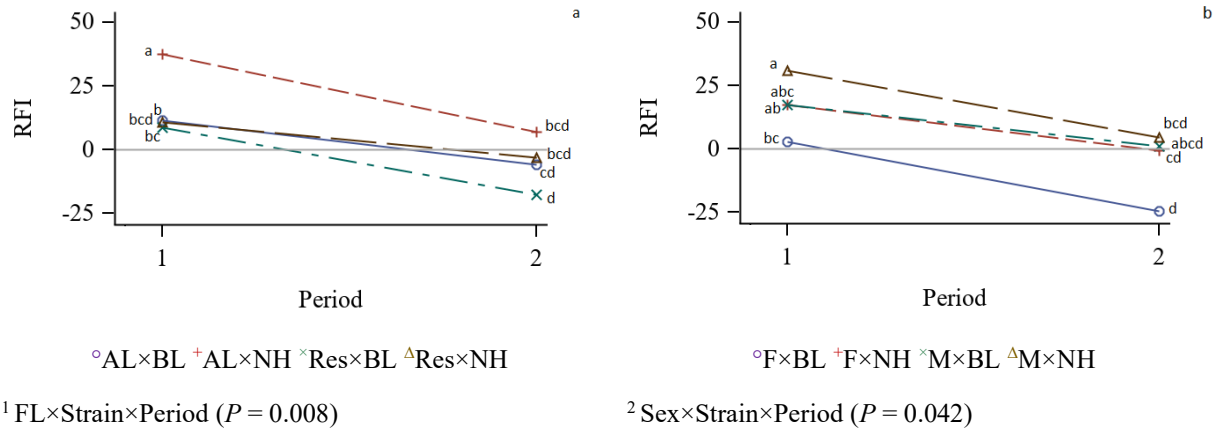


Figure 3. 1. Residuals for a) Fixed-effects growth model [1] $BW = Wm \times exp^{-exp^{-b(t-t_{inf})}}$; b) Nonlinear mixed-effects growth model [2] including rate of maturing random coefficient (b_u); c) Nonlinear mixed-effects growth model [3] including mature BW random coefficient (Wm_u); d) Nonlinear mixed-effects growth model [4] including both Wm_u and b_u, for mixed-sex New Hampshire and Brown Leghorn heritage chickens.



°AL×BL +AL×NH ×Res×BL ΔRes×NH

[1] FL×Strain×Period ($P = 0.008$)

°F×BL +F×NH ×M×BL ΔM×NH

[2] Sex×Strain×Period ($P = 0.042$)

Figure 3. 2. Interaction effect of feeding level, strain, and period (Panel a), and sex, strain, and period (Panel b) on RFI value of New Hampshire and Brown Leghorn heritage chickens under ad libitum and restricted feed intake pair-fed treatments.

[1] FL: Feeding level; AL: Ad libitum; Res: Feeding level; AL: Ad libitum; Res: Restricted from 12-17 wk of age at 95% of the AL intake of a paired bird closest in BW; BL: Brown Leghorn; NH: New Hampshire.

[2] F: Female; M: Male.

4.0 Chapter 4: Timing of Growth Affected Broiler Breeder Feeding Motivation and Reproductive Traits

4.1 Abstract

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

motivation index by 1.6 and 0.8 visits/meal during rearing and laying phase, respectively. Earlier pubertal growth showed prominent effects on the reproductive performance.

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

4.2 Introduction

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

Reproductive performance is compromised by both unrestricted BW in female breeders (Robinson et al., 1993; Heck et al., 2004) and severe feed restriction (Wilson and Harms, 1986).

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

Potential approaches to reduce the intensity of feed restriction in broiler breeders have been investigated in various studies through diet dilution (Zuidhof et al., 1995; Savory and Lariviere, 2000), relaxed feed restriction (Hocking et al., 2002a; Bruggeman et al., 2005; Zukiwsky et al., 2021), and introduction of alternative genetic stock (Heck et al., 2004; Bruggeman et al., 2005). Hocking et al. (2002a) found that increasing target BW by 20% at 18 wk of age did not affect egg or chick production. They reported no difference in the welfare traits (measure of immune function, physiological indices of stress, and behavioral changes) of the hens, which indicated no real benefit of the relaxed feed restriction protocols tested in their studies (Hocking et al., 2001, 2002b). Zukiwsky et al. (2021) increased broiler breeders target BW gain during prepubertal and pubertal phases incrementally up to 22.5% above the recommended BW target. They included a group of unrestricted birds in their study. Some of the unrestricted pullets commenced egg production 2 wk prior to photostimulation. These results strongly suggest that body composition and metabolic status have a role in triggering sexual

maturation. Notably, the authors reported that relaxing growth restriction by up to 22.5% above the recommended BW target decreased hunger in hens during laying phase but not in pullets during the rearing phase. Hadinia et al (2020) increased broiler breeder dietary energy by 302 kcal/kg (from 2,807 to 3,109 kcal/kg of diet) from 22 to 26 wk of age. The percentage of birds which commenced laying was 100% in the high ME intake treatment and 30% in the low ME intake treatment. They concluded higher ME intake advanced the activation of hypothalamus-pituitary-gonadal axis, stimulated reproductive hormone levels, and increased lipid deposition in the body of high ME intake treatment group.

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

4.3 Materials and Methods

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

4.3.1 Experimental Design

The current experiment was conducted as a randomized controlled trial. A total of 40 female Ross 708 broiler breeder pullets were equally and randomly assigned to 10 growth trajectories (Figure 4.1). Growth trajectories were designed with 2 levels of the amount of early growth and 5 levels of timing of growth around puberty. Coefficients of growth parameters for

breeder-recommended growth trajectory were estimated using a 3-phase Gompertz model fit to the breeder-recommended target BW (Aviagen, 2016). The model (Zuidhof, 2020) had the form:

$$BW_t = \sum_{i=1}^{i=3} g_i exp^{-exp^{-b_i(t-I_i)}} + \varepsilon_t$$

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

4.3.2 Animals and Management

The pullets (n=40) were housed in a single pen containing 2 PF systems, from hatch to 43 wk of age at a stocking density of 3.0 birds per m^2. All birds were fed a commercial diet: starter (crumble; ME 2,726 kcal/kg, 21.0% CP, 1.0% Ca, and 0.45% available P) from hatch to d 34; grower (mash; ME 2,799 kcal/kg, CP 15.0%, 0.79% Ca, and 0.44% available P) from d 35 to d 179; and breeder diet (crumble; ME 2,798 kcal/kg, 15.3% CP, 3.30% Ca, and 0.38% available P)

from 180 d onward. Water was provided ad libitum throughout the experiment. The photoschedule was 24L:0D (100 lx) from d 0 to 3 then reduced to 8L:16D (15 lx) on d 4. Pullets were photostimulated at wk 22 by increasing the photoperiod to 11L:13D (20 lx); to 12L:12D (25 lx) at wk 23, then at wk 24 to 13L:11D (50 lx) for the remainder of the experiment. Each PF station had 5 green LED lights (2 lx) that illuminated the inside for 24 h/d so that birds could see their way through the station during the scotophase, without causing photostimulation (Rodriguez, 2017). Room temperature was maintained at 33°C during the first 2 d, and from d 3 onwards temperature was gradually reduced to 20°C by wk 5. A trap nest with 8 nesting sites and a nest box with 8 nesting sites equipped with RFID readers which identified and weighed eggs of individual hens were installed in the room at 14 wk of age; thus, the pullets had the chance to adapt to the nesting system prior to the onset of lay.

All birds were fed individually using a PF system (Zuidhof et al., 2019) that imposed appropriate feed intake levels to achieve the target growth trajectories of each individual bird. Each PF station consisted of 2 motorized entry doors, a sorting and feeding stage, a feeder, and a ramp giving access to the sorting stage. In addition to feed availability from the PF station, supplemental feed was provided on paper plates located around the ramp, on the ramp, and throughout the station, which were gradually removed over the first wk, to encourage chicks to enter the station individually to reach the feeder. During the training period (first 2 wk), the chicks were placed on the ramp, sorting stage, and feeding stage to get trained to use the PF stations. At 14 d of age each bird was equipped with a wing band containing a radio frequency identification (RFID) transponder to be recognized individually by the PF system. Birds were individually weighed by the PF system in real-time. The treatment BW trajectories were uploaded to the PF system on 14 d of age. The PF system provided access to a small meal for 60

s if the individual bird's real-time BW was equal or less than the pre-programmed target BW; otherwise, the system gently ejected the birds from the PF station. The chicks were weighed manually daily during the first 3 wk to confirm growth and adoption to the PF system. Feed intake and visit frequency were checked daily to ensure all birds were accessing the PF system. Chicks were provided with additional training to adapt to individual feeding within the feeding station if their BW gain was less than 5 g, FI was less than 2 g, or had less than 3 station visits over the previous 24 h. The birds had access to the PF system 24 hours per day throughout the experiment.

4.3.3. Data Collection

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

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

skinfold. The dial was read to the nearest 0.50 mm, 1 to 2 seconds after the spring tension had been fully applied. Body fat as a percentage of BW was estimated using the following model. $Body\ fat\ (\%) = 24.83 + 6.75\ (\ln skinfold) - 3.87\ BW$, where skinfold was abdominal skinfold thickness (cm), and BW was measured in kg. The model was created using data from Ross, Avian, and Sex-Links strains with an $R^2 = 0.63$ (Latshaw and Bishop, 2001).

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

4.3.4 Statistical Analysis

Analysis of covariance was conducted on hen-day egg production, EW, EM, station visit frequency, meal frequency, meal size, and visit:meal ratio variables using the MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC), with g_1 and time period as discrete sources of variation, and I_2 as a continuous predictor variable. Period was included in the model as a

random effect with individual bird as the subject to account for within-bird variation. The same analysis was conducted on shank length, estimated body fat, AFE, BW at photostimulation (**BWPS**), and BW at first egg (**BWFE**) without including period in the analysis. Pairwise differences between means within each period were determined using Tukey's HSD test and were reported as different when $P \leq 0.05$. Trends were reported where $0.05 < P \leq 0.10$.

4.4 Results and Discussion

Standard coefficients of growth parameters in the 3-phasic Gompertz model were estimated for Ross 708 breeder-recommended BW trajectory (Table 4.1; Figure 4.1). Then g_1 was increased by 10% to create High g_1 BW trajectories (Table 4.1). The breeder recommended I_2 at 22.29 wk of age predicted accumulation of 90% of the total growth for the pubertal phase in approximately 20 wk, from 17 to 37 wk of age. Pubertal inflection point was advanced in both Standard and High g_1 treatments creating inflection points that varied by 1.1 wk (7.8 d) in the range of 17.82 to 22.29 wk of age. Correspondingly, the predicted timeframe for accumulation of 90% of the total pubertal growth advanced by 7.8 d with each 5% advancement of I_2 (Table 4.1; Figure 4.2).

4.4.1 Body Weight

Body weight was similar across BW trajectories from 3 to 6 wk of age (Table 4.2). Target BW might have not diverged enough among BW trajectories (Figure 4.1) to detect significant differences in bird BW by 6 wk of age. High g_1 pullets had a greater average BW than that of their Standard g_1 counterparts from 7 to 8 wk of age. However, earlier I_2 did not increase BW within the Standard g_1 and High g_1 treatments by 8 wk of age. Pullet BW started to diverge within the Standard g_1 and High g_1 treatments at 9 wk of age due to earlier I_2 (earlier pubertal

growth). Increasing g_1 by 10% increased BWPS for High g_1 hens by 6.4% (167 g) compared to that of the Standard g_1 hens ($P < 0.001$, Table 4.3). For every week earlier I_2, BWPS and BWFE increased by 126 g ($P < 0.001$) and 94 g ($P < 0.001$). After 36 wk of age, there were no differences among bird BW within the Standard g_1 and High g_1 treatments (Table 4.2) as the target growth trajectories started to converge (Figure 4.1).

4.4.2 Shank Length and Body Fat

Shank length and estimated body fat were used as proxies for body frame size and body composition, respectively. Advancing the inflection point of the second (pubertal) growth phase increased shank length at 24 wk of age by 0.038 and 1.495 mm/wk within the Standard g_1 and High g_1 treatments, respectively ($P = 0.046$, Table 4.4). Renema et al. (2007) noted that feed restriction can limit shank length throughout the rearing phase. Achieving adequate body frame development threshold provides the bird the foundation for a successful laying cycle (Shi et al., 2020). Increasing g_1 by 10% did not affect the estimated body fat. For every week of earlier pubertal growth, estimated body fat increased by 0.38% ($P = 0.013$, Table 4.4). It was shown that carcass fat at sexual maturity is between 11-15% of total BW (Joseph et al., 2000; Renema et al., 2007), which is not consistent with the estimated body fat in the current study (8.0 ± 0.4 and 8.5 ± 0.4% for the Standard g1 and High g1 treatments, respectively). This might be due to low body fat in Ross 708 strain (Renema et al., 2007) and the fact that body fat has decreased in modern broiler breeders (Caldas et al., 2018). To commence egg production and support adequate reproductive performance in broiler breeders, a minimum percentage of body fat is required (Sun and Coon, 2005; de Beer and Coon, 2009; van Emous et al., 2013). In the current study all birds reached the sexual maturity and commenced egg laying; thus, the minimum body fat threshold is likely below 8%.

4.4.3 Age at First Egg

Standard g_1 and High g_1 hens commenced lay at almost the same age (176 d, Table 4.3). Age at first egg advanced by 0.49 d/wk of earlier I_2 ($P = 0.046$, Table 4.3). This might be because birds with earlier pubertal growth had higher estimated body fat, as a measure of body composition, and longer shank length, as a measure of body frame size, compared to their counterparts with standard I_2 (Table 4.4). These birds may have reached the BW and body composition thresholds required for onset of lay because of earlier pubertal growth. Thus, achieving those thresholds may have provided sufficient metabolic triggers for sexual maturation. Extra ME and nutrients at this time can advance the sexual maturation process in broiler breeder individuals by advancing the activation of the hypothalamus-pituitary-gonadal axis and increasing body lipid deposition (Renema et al., 1999; Hadinia et al., 2020). However, Renema et al. (2007) did not find an advancement in AFE when they increased 12-wk target BW by 150 and 200% and photostimulated the birds at 22 wk of age. We previously reported that there is individual variation in the thresholds for sexual maturity because each bird might have a unique BW threshold to reach sexual maturity (Zukiwsky et al., 2021).

4.4.4 Egg Production, Egg Mass, and Egg Weight

Compared to the Standard g_1 hens High g_1 hens produced one more egg/hen/period ($P = 0.013$, Table 4.5) and 2.95 g/d greater EM ($P = 0.022$). High and Standard g_1 hens produced 110 and 105 eggs/hen throughout the laying phase, respectively ($P = 0.047$; data not shown). Increasing BW by 20% (430 g) at 20 wk of age increased number of eggs per hen housed (Ekmay et al., 2012). In the current study for every week of earlier I_2, BW at 20 wk of age increased by 6.5%; number of eggs/hen increased by 0.33 egg/hen for the High g_1 treatment and decreased by 0.27 egg/hen and for the Standard g_1 treatment ($P = 0.021$); EM increased by 0.916

g/d for the High g_1 treatment and decreased by 0.29 g/d for the Standard g_1 treatment (P = 0.040). As the decision of the PF system to feed birds was based on their target BW, the High g_1 birds received more feed during the laying phase compare to their Standard g_1 counterparts. Thus, after meeting their maintenance ME requirements, their egg production potential would not have been less limited by restricted ME intake, compared to the Standard g_1 hens. To our knowledge, this is the first time to investigate the effect of a systematically designed pubertal growth inflection point on EW and EM.

Increasing g_1 by 10% (160 g at 20 wk of age, Table 4.2) did not affect EW. This is in agreement with the results of previous studies where increasing target BW by 8% (158 g) at 20 wk of age (Fattori et al., 1991), 20% (365 g) at 18 wk of age (Hocking et al., 2002b), 8% (163 g) at 20 wk of age (van Emous et al., 2013), or 16% (370 g) at 20 wk of age (Gous and Cherry, 2004) did not affect EW. The reason for the lack of an effect of increasing g_1 on EW maybe because the difference in BW at 20 wk of age (160 g) between the Standard and High g_1 birds was not large enough to affect the average EW. However, increasing target BW in other research by 21% (338 g) or 13% (229 g) at 20 wk of age were sufficient to increase EW (Renema et al., 2001; Sun and Coon, 2005). Egg weight increased by 0.27 g/week of earlier I_2 (P = 0.036, Table 4.5). The difference in BW between birds with standard I_2 (22.29 wk) and those with I_2-20% (17.82 wk) was 554 g within both Standard and High g_1 hens at 20 wk of age (Table 4.2). This large difference in BW due to earlier pubertal growth might have increased EW in hens with advanced I_2 but did not persist once the BW trajectories started merging at 36 wk of age (Figure 4.1). The effect of BW trajectories on EW towards later phase of laying is not clear as the current study analysis was conducted until 42 wk of age.

4.4.5 Feeding Motivation

The frequency of daily station visits, visit:meal ratio, and meal size could all be indicators of feeding motivation. During the rearing phase, Standard g_1 pullets had approximately 7 more daily station visits compared to the High g_1 pullets ($P = 0.005$, Table 4.6), which would be consistent with a higher degree of hunger in the Standard g_1 birds. For every week that I_2 was advanced, the station visit frequency decreased by 2.55 visits in the Standard g_1 pullets and increased by 1.08 visits in the High g_1 group. Birds with earlier I_2 started to accumulate pubertal gain earlier than those with standard I_2 resulting in a lower degree of feed restriction. Thus, it is possible that those Standard g_1 birds with earlier I_2 were less hungry and less motivated to enter the feeding station to seek feed compared to their counterparts with standard I_2. High g_1 pullets might have approached a point of satiety because of having 10% higher g_1; thus, earlier I_2 did not decrease their daily station visits. During the laying phase, the frequency of daily station visits was not affected by g_1 but was increased by 0.83 and 4.97 visits/d/wk of earlier I_2 ($P = 0.002$).

Increasing g_1 by 10% increased meal frequency during rearing ($P < 0.001$, Table 4.6) and laying phase ($P = 0.041$, Table 4.7) because of increased target BW in the High g_1 birds to support maintenance requirements, prepubertal growth (muscle and skeletal development) during rearing, pubertal growth (development of reproductive tract and fat deposition) towards the end of rearing, and egg production throughout the laying phase. Meal frequency increased by 0.34 meal/wk of earlier I_2 during the rearing phase ($P < 0.001$, Table 4.6). This was expected, as feed restriction is reportedly most severe from 8 to 16 wk of age when broiler breeders are restricted 25 to 30% of the intake of unrestricted birds (de Jong and Jones, 2006). Thus, increasing BW target by advancing I_2 decreased the level of feed restriction as the birds had access to feed based on their BW. This is in line with an increase in ADFI by 3.9 g/d/wk of earlier I_2 ($P < 0.001$).

However, for every week of earlier I_2, meal frequency tended to decrease by 0.25 meals/d during the laying phase ($P = 0.055$, Table 4.7).

Feeding motivation index was defined as the visit:meal ratio indicating the feed seeking motivation, driven by the number of meals allowed. Feeding motivation index for the Standard g_1 and High g_1 birds was 8.6 and 7.0 visits/meal during the rearing phase (Table 4.6) and 4.8 and 4.0 visits/meal during the laying phase (Table 4.7), respectively. Thus, High g_1 birds had 1.6 and 0.8% lower feeding motivation index than that of the Standard g_1 birds during the rearing and laying phase, respectively. Earlier I_2 reduced feeding motivation index during the rearing phase by 0.75 and 0.16 visits/meal in the Standard g_1 and High g_1 pullets, respectively ($P = 0.038$, Table 4.6). A lower reduction in feeding motivation index of High g_1 pullets compared to their Standard g_1 counterparts indicates that increasing g_1 by 10% had already decreased their hunger in such a way that earlier I_2 (further release in growth restriction) just had a minor effect on alleviating their hunger. These results are in line with Savory and Lariviere (2000) who investigated broiler breeder feeding motivation using an operant conditioning system during the rearing phase. The birds were receiving feed as a reward after pecking at a disc implemented in the operant system. The authors measured the number of operant responses in 12 min as a proxy of feeding motivation and found a positive relationship between feed motivation and suppression of growth rate. Their study showed that the number of operant responses decreased by 63, 45, 57, and 62 times per each kg increase in BW at 8, 10, 12, and 14 wk of age, respectively. However, the results of the current study during the rearing phase are in contrast with results from Zukiwsky et al. (2021) who did not observe a decrease in feed seeking behavior during the rearing phase as BW increased up to 22.5% above the recommended BW target. In fact, they used daily station visits as an indicator of feed seeking behaviour and did not account for the

meal frequency by calculating the visit:meal ratio. In the current analysis, the feeding motivation index accounted for the meal frequency. Earlier pubertal growth reduced feeding motivation index for both Standard g_1 and High g_1 pullets. However, using daily station visit frequency on its own showed an increase in "feeding motivation" for those High g_1 pullets with earlier I_2 compared with their counterparts with a standard I_2. Therefore, it could be hypothesized that visit:meal ratio might be a better indicator of feeding motivation compared to daily station visit frequency.

Feeding motivation is affected by both external and internal factors. For instance, feeding motivation in broiler breeders is affected by both increased appetite because of genetic selection (internal) and the availability and allocation of feed in the environment (external). Every day a hen produced an egg, BW of the hen was reduced by the weight of the egg, so the hen qualified for additional feed allocation through the PF system, as the PF feed allocation decision was based on BW. During the laying phase, feeding motivation index increased by 0.33 visits/meal/wk of earlier I_2 ($P < 0.001$). As the birds with earlier I_2 commenced egg production earlier than those with standard I_2 ($P = 0.046$, Table 4.3), they qualified for additional feed allocation as an external feeding motivation. It could have motivated the birds with earlier I_2 to seek feed from the PF system leading to an increased visit:meal ratio.

Meal size might also be an indicator of hunger and feeding motivation. A larger meal size was related to a faster feed intake rate, as birds had 60 s to eat off the feeder before being ejected from the PF system. Meal size increased by age ($P < 0.001$, Table 4.6 and 4.7) but was not affected by the g_1 treatment during rearing and laying phases. During the rearing phase meal size increased by 0.08 and 0.03 g/visit/wk of earlier I_2 for the Standard g_1 and High g_1 pullets, respectively ($P = 0.038$, Table 4.6). This corresponds with an increase in ADFI by 3.9 g/d/wk of

earlier I_2 to fulfill nutrient requirements associated with weight gain ($P < 0.001$). Furthermore, High g_1 pullets had 5.1 g/d greater ADFI than that of Standard g_1 pullets ($P < 0.001$), which was because of decreased feed restriction in the High g_1 pullets. During the laying phase, meal size tended to increase by 0.18 g/visit/wk of earlier I_2 ($P = 0.068$, Table 4.7). Earlier I_2 decreased ADFI by 1.3 g/d/wk for the Standard g_1 hens and increased it by 1.2 g/d/wk for the High g_1 birds ($P < 0.001$). This might have been due to higher station visit frequency with earlier I_2 for High g_1 birds (4.97 visit/wk) compared to that of Standard g_1 (0.83 visit/wk) hens during the laying phase (Table 4.7).

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

4. 5 Acknowledgment

Funding from Alberta Agriculture and Forestry (Edmonton, AB), Poultry Innovation Partnership (Edmonton, AB), and technical assistance from Chris Ouellette is gratefully

acknowledged. In-kind support for the precision feeding system was provided by Xanantec Technologies, Inc. (Edmonton, AB, Canada).

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4.7 Tables

Table 4. 1. Estimated coefficients of a 3-phase Gompertz model[1] used to generate target BW trajectories[2] for Ross 708 broiler breeders.

	BW trajectory									
	Standard g_1					High g_1				
Growth parameter	I_2-0%	I_2-5%	I_2-10%	I_2-15%	I_2-20%	I_2-0%	I_2-5%	I_2-10%	I_2-15%	I_2-20%
n [3]	4	4	4	4	4	4	4	4	4	4
Mortality	0	0	0	1	0	0	0	1	1	0
g_1 (kg)	1.880	1.880	1.880	1.880	1.880	2.068	2.068	2.068	2.068	2.068
b_1	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147	0.147
I_1 (wk)	7.30	7.30	7.30	7.30	7.30	7.30	7.30	7.30	7.30	7.30
g_2 (kg)	1.696	1.696	1.696	1.696	1.696	1.696	1.696	1.696	1.696	1.696
b_2	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208
I_2 (wk)	22.29	21.16	20.05	18.94	17.82	22.29	21.16	20.05	18.94	17.82
g_3 (kg)	0.451	0.451	0.451	0.451	0.451	0.451	0.451	0.451	0.451	0.451
b_3	0.094	0.094	0.094	0.094	0.094	0.094	0.094	0.094	0.094	0.094
I_3 (wk)	54.85	54.85	54.85	54.85	54.85	54.85	54.85	54.85	54.85	54.85

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

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

[3] n was number of birds grown in each growth trajectory.

Table 4. 2. Effect of BW trajectory [1] (W) and time period on BW during rearing and laying phases in Ross 708 broiler breeders.

		BW trajectory																			
		Standard g_1										High g_1									
		I_2-0%		I_2-5%		I_2-10%		I_2-15%		I_2-20%		I_2-0%		I_2-5%		I_2-10%		I_2-15%		I_2-20%	
Phase	Period	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM
	— wk —	— g —																			
Rearing	3	341	22.9	332	22.9	302	22.9	320	26.5	328	22.9	360	22.9	320	26.5	313	26.5	296	26.5	350	22.9
	5	553	15.7	555	15.7	538	15.7	554	18.2	560	15.7	610	15.7	595	18.2	598	18.2	544	18.2	581	15.7
	7	761^{b}	6.5	760^{b}	6.5	760^{b}	6.5	760^{b}	7.5	760^{b}	6.5	836^{a}	6.5	834^{a}	7.5	834^{a}	7.5	810^{a}	7.5	832^{a}	6.5
	9	957^{d}	1.0	955^{d}	1.0	957^{d}	1.0	960^{d}	1.2	967^{c}	1.0	1,051^{b}	1.0	1,052^{b}	1.2	1,052^{b}	1.2	1,055^{ab}	1.2	1,061^{a}	1.0
	11	1,136^{g}	1.0	1,136^{g}	1.0	1,142^{g}	1.0	1,161^{f}	1.2	1,198^{e}	1.0	1,248^{d}	1.0	1,250^{d}	1.2	1,257^{c}	1.2	1,274^{b}	1.2	1,310^{a}	1.0
	13	1,295^{i}	1.9	1,312^{h}	1.9	1,338^{g}	1.9	1,395^{f}	2.2	1,470^{c}	1.9	1,425^{e}	1.9	1,438^{d}	2.2	1,469^{c}	2.2	1,525^{b}	2.2	1,605^{a}	1.9
	15	1,462^{j}	1.3	1,513^{i}	1.3	1,587^{h}	1.3	1,687^{e}	1.6	1,811^{c}	1.3	1,604^{g}	1.3	1,652^{f}	1.6	1,729^{d}	1.6	1,829^{b}	1.6	1,955^{a}	1.3
	17	1,673^{j}	0.9	1,771^{i}	0.9	1,888^{g}	0.9	2,022^{e}	1.1	2,166^{c}	0.9	1,826^{h}	0.9	1,923^{f}	1.1	2,041^{d}	1.1	2,178^{b}	1.1	2,321^{a}	0.9
	19	1,945^{j}	1.1	2,078^{i}	1.1	2,218^{g}	1.1	2,362^{e}	1.3	2,499^{c}	1.1	2,106^{h}	1.1	2,237^{f}	1.3	2,378^{d}	1.3	2,523^{b}	1.3	2,660^{a}	1.1
	21	2,255^{j}	1.1	2,398^{i}	1.1	2,534^{g}	1.1	2,665^{e}	1.3	2,781^{c}	1.1	2,422^{h}	1.1	2,563^{f}	1.3	2,703^{d}	1.3	2,833^{b}	1.3	2,948^{a}	1.1
Laying	23	2,556^{j}	2.8	2,687^{i}	2.8	2,804^{g}	2.8	2,909^{e}	3.3	3,000^{c}	2.8	2,730^{h}	2.8	2,848^{f}	3.3	2,975^{d}	3.3	3,083^{b}	3.3	3,164^{a}	2.8
	25	2,813^{f}	16.2	2,922^{e}	16.2	2,989^{de}	16.2	3,094^{cd}	18.7	3,163^{bc}	16.2	2,996^{de}	16.2	3,029^{de}	18.7	3,187^{bc}	18.7	3,270^{ab}	18.7	3,328^{a}	16.2
	27	3,018^{f}	18.4	3,099^{ef}	18.4	3,150^{de}	18.4	3,231^{bcd}	21.3	3,280^{bc}	18.4	3,195^{cde}	18.4	3,207^{cde}	21.3	3,346^{ab}	21.3	3,412^{a}	21.3	3,439^{a}	18.4
	29	3,177^{e}	16.7	3,225^{de}	16.7	3,283^{cd}	16.7	3,324^{cd}	19.2	3,364^{bc}	16.7	3,356^{c}	16.7	3,332^{cd}	19.2	3,468^{ab}	19.2	3,503^{a}	19.2	3,531^{a}	16.7
	31	3,293^{f}	13.9	3,325^{ef}	13.9	3,365^{def}	13.9	3,395^{cde}	16.1	3,426^{cd}	13.9	3,466^{bc}	13.9	3,445^{cd}	16.1	3,558^{ab}	16.1	3,578^{a}	16.1	3,597^{a}	13.9
	33	3,382^{f}	13.5	3,402^{ef}	13.5	3,431^{def}	13.5	3,446^{def}	15.6	3,468^{cde}	13.5	3,552^{bc}	13.5	3,512^{cd}	15.6	3,619^{ab}	15.6	3,638^{ab}	15.6	3,651^{a}	13.5

35	3,436^{d}	18.5	3,445^{d}	18.5	3,471^{d}	18.5	3,483^{cd}	21.4	3,485^{d}	18.5	3,604^{abc}	18.5	3,544^{bcd}	21.4	3,658^{ab}	21.4	3,671^{ab}	21.4	3,680^{a}	18.5
37	3,478^{b}	17.8	3,483^{b}	17.8	3,496^{b}	17.8	3,505^{b}	20.5	3,497^{b}	17.8	3,646^{a}	17.8	3,595^{ab}	20.5	3,691^{a}	20.5	3,694^{a}	20.5	3,697^{a}	17.8
39	3,510^{b}	22.0	3,506^{b}	22.0	3,517^{b}	22.0	3,528^{b}	25.4	3,536^{b}	22.0	3,677^{a}	22.0	3,596^{ab}	25.4	3,711^{a}	25.4	3,721^{a}	25.4	3,714^{a}	22.0
41	3,530^{b}	25.0	3,532^{b}	25.0	3,533^{b}	25.0	3,548^{b}	28.8	3,558^{b}	25.0	3,661^{ab}	25.0	3,581^{ab}	35.3	3,733^{a}	28.8	3,732^{a}	28.8	3,733^{a}	25.0

Source of variation	*P-value*
Rearing W	< 0.001
Period	< 0.001
W×Period	< 0.001
Laying W	< 0.001
Period	< 0.001
W×Period	< 0.001

[1] A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. BW trajectories were designed with two levels of prepubertal BW gain (g_1) coefficient and 5 levels of pubertal growth phase inflection point (I_2) coefficient. g_1 was estimated from the breeder-recommended standard BW gain (Standard g_1) target, or 10% higher (High g_1). Second growth phase (pubertal) inflection point (I_2) was advanced such that I_2-0% = 22.29 wk, I_2-5% = 21.16 wk, I_2-10% = 20.05 wk, I_2-15% = 18.94 wk, I_2-20% = 17.82 wk.

[a – j] Means within rows with no common superscript differ ($P < 0.05$).

Table 4. 3. Effects of prepubertal BW gain and pubertal growth inflection on BW at photostimulation (BWPS) and BW at first egg (BWFE) of Ross 708 broiler breeder pullets.

Effect [1]	g_1	AFE [2]	SEM	BWPS	SEM	BWFE	SEM
		day		g			
g_1	Standard g_1	175.7	1.3	2,614[b]	2.42	2,943[b]	21.96
	High g_1	175.6	1.4	2,781[a]	2.64	3,112[a]	23.94
		day/wk		g/wk			
I_2		0.49	0.83	126	1.52	94	13.75
$I_2 \times g_1$	Standard g_1	0.49	0.83	126	1.52	94	13.75
	High g_1	1.48	1.19	190	2.17	59	19.71
Source of variation		*P-value*					
g_1		0.22		< 0.001		0.58	
I_2		0.046		< 0.001		< 0.001	
$I_2 \times g_1$		0.22		0.53		0.33	

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

[2] AFE: Age at first egg.

[a – b] Means within columns with no common superscript differ ($P < 0.05$).

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

Effect [1]	g_1	Shank length [2]	SEM	Body fat [3]	SEM
		mm		%	
g_1	Standard g_1	98.4	0.8	8.04	0.38
	High g_1	99.9	0.8	8.47	0.38
		mm/wk		%/wk	
I_2		-0.038	0.511	-0.38	0.24
$I_2 \times g_1$	Standard g_1	-0.038	0.511	-0.38	0.24
	High g_1	-1.495	1.216	-0.53	0.59
Source of variation		*P-value*			
g_1		0.19		0.44	
I_2		0.036		0.013	
$I_2 \times g_1$		0.046		0.67	

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

[2] Shank length = tibiotarsus measured from top of flexed hock joint to bottom of footpad.

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

Table 4. 5. Effects of prepubertal BW gain and pubertal growth inflection on egg weight (EW), egg mass (EM), and number of eggs during 4 wk periods from 23 to 42 wk of age of Ross 708 broiler breeder hens.

Effect [1]	g_1	Period (wk)	EW	SEM	EM	SEM	Egg	SEM
			—— g ——		—— g/d ——		– Egg/hen/period –	
g_1	Standard g_1		59.2	0.4	42.73^{b}	0.70	20^{b}	0.3
	High g_1		60.0	0.4	45.68^{a}	0.76	21^{a}	0.4
Period		23 to 26	52.8^{d}	0.5	20.15^{c}	1.70	11^{d}	0.9
		27 to 30	56.8^{c}	0.5	52.07^{a}	0.86	26^{a}	0.4
		31 to 34	59.6^{b}	0.5	44.29^{b}	1.06	21^{c}	0.5
		35 to 38	64.5^{a}	1.1	53.73^{a}	1.40	24^{ab}	0.6
		39 to 42	64.1^{a}	0.8	50.78^{a}	0.90	23^{bc}	0.4
			— g/wk ——		— g/d/wk —		— Egg/hen/wk —	
I_2			-0.27	0.21	0.29	0.40	0.27	0.18
$I_2 \times g_1$	Standard g_1		-0.27	0.21	0.29	0.40	0.27	0.18
	High g_1		-0.37	0.52	-0.916	0.99	-0.33	0.44

Source of variation	*P-value*		
g_1	0.13	0.022	0.013
I_2	0.036	0.29	0.13
$I_2 \times g_1$	0.75	0.040	0.021
Period	< 0.001	< 0.001	< 0.001

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

[a – d] Means within columns with no common superscript differ ($P < 0.05$).

Table 4. 6. Effects of prepubertal BW gain and pubertal growth inflection on the station visit frequency, meal frequency, feeding motivation index, and meals size during rearing phase of Ross 708 broiler breeder pullets.

Effect [1]	g_1	Period	Visits	SEM	Meals	SEM	Feeding motivation index [2]	SEM	Meal size	SEM	ADFI	SEM
		– wk –	—— visit ——		— meals —		———— visits/meal ————		—— g/visit ——		— g/day —	
g_1	Standard g_1		53.7 ^{a}	1.9	7.1^{b}	0.1	8.6^{a}	0.3	9.0	0.1	62.2^{b}	0.8
	High g_1		46.0 ^{b}	2.1	7.6^{a}	0.1	7.0^{b}	0.4	9.2	0.1	67.3^{a}	0.9
Period		3 to 6	34.9^{c}	3.2	8.4^{a}	0.3	6.1^{c}	0.7	7.0^{d}	0.3	50.6^{c}	2.8
		7 to 10	63.1^{a}	4.0	6.2^{c}	0.2	11.0^{a}	0.7	8.6^{c}	0.2	53.3^{c}	2.0
		11 to 14	55.7^{ab}	3.6	6.6^{c}	0.1	8.9^{ab}	0.6	8.7^{c}	0.1	56.2^{c}	0.5
		15 to 18	49.5^{b}	2.6	7.2^{b}	0.1	7.3^{bc}	0.5	10.3^{b}	0.1	74.0^{b}	1.0
		19 to 22	46.1^{b}	2.5	8.3^{a}	0.2	5.9^{c}	0.4	11.0^{a}	0.2	89.5^{a}	1.0
			– visit/wk –		– meals/wk –		——— visits/meal/wk ———		— g/visit/wk ——		– g/day/wk –	
I_2			2.55	1.15	-0.34	0.05	0.75	0.19	-0.08	0.06	-3.9	0.3
$I_2 \times g_1$	Standard g_1		2.55	1.15	-0.34	0.05	0.75	0.19	-0.08	0.06	-3.9	0.3
	High g_1		-1.08	1.65	-0.34	0.08	0.16	0.47	-0.03	0.09	-3.4	0.8
Source of variation			——————————————— *P-value* ———————————————									
g_1			0.005		< 0.001		< 0.001		0.17		< 0.001	
I_2			0.37		< 0.001		0.001		0.21		< 0.001	
$I_2 \times g_1$			0.029		0.99		0.038		0.038		0.35	
Period			< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	

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

[2] Feeding motivation index was defined as daily station visit:meal ratio.

[a – c] Means within columns with no common superscript differ ($P < 0.05$).

Table 4. 7. Effects of prepubertal BW gain and pubertal growth inflection on the station visit frequency, meal frequency, motivation index, and meals size during laying phase of Ross 708 broiler breeder hens.

Effect [1]	g_1	Period	Visits	SEM	Meals	SEM	Feeding motivation index [2]	SEM	Meal size	SEM	ADFI	SEM
		– wk –	—— visit ——		— meals —		———— visits/meal ————		—— g/visit ——		— g/day —	
g_1	Standard g_1		37.8	1.5	9.4^{b}	0.2	4.8^{a}	0.2	15.1	0.2	132.9^{b}	0.9
	High g_1		34.4	1.6	10.0^{a}	0.2	4.0^{b}	0.2	15.2	0.3	141.0^{a}	0.9
Period		23 to 26	45.0^{a}	2.8	10.8^{a}	0.4	5.4^{a}	0.4	9.7^{d}	0.2	101.1^{d}	1.9
		27 to 30	33.6^{b}	2.2	11.6^{a}	0.4	3.2^{b}	0.3	13.8^{c}	0.4	147.6^{b}	1.2
		31 to 34	37.9^{ab}	2.4	9.2^{b}	0.3	5.2^{a}	0.4	18.5^{a}	0.5	159.7^{a}	1.8
		35 to 38	31.4^{b}	2.4	8.2^{b}	0.3	4.1^{ab}	0.3	17.5^{ab}	0.5	138.3^{c}	1.4
		39 to 42	32.5^{b}	2.1	8.9^{b}	0.3	4.2^{ab}	0.3	16.2^{b}	0.5	138.1^{c}	1.0
			– visit/wk –		– meals/wk –		———— visits/meal/wk ————		— g/visit/wk ——		– g/day/wk –	
I_2			-0.83	0.90	0.25	0.12	-0.33	0.12	-0.18	0.12	1.3	0.5
$I_2 \times g_1$	Standard g_1		-0.83	0.90	0.25	0.12	-0.33	0.12	-0.18	0.12	1.3	0.5
	High g_1		-4.97	1.29	0.08	0.29	-0.54	0.29	-0.14	0.18	-1.2	0.7
Source of variation			———————————————— *P-value* ————————————————									
g_1			0.11		0.041		0.010		0.70		< 0.001	
I_2			< 0.001		0.055		< 0.001		0.068		0.88	
$I_2 \times g_1$			0.002		0.34		0.22		0.83		< 0.001	
Period			0.002		< 0.001		< 0.001		< 0.001		< 0.001	

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

[2] Feeding motivation index was defined as daily station visit:meal ratio.

[a – c] Means within columns with no common superscript differ ($P < 0.05$).

4.8 Figures

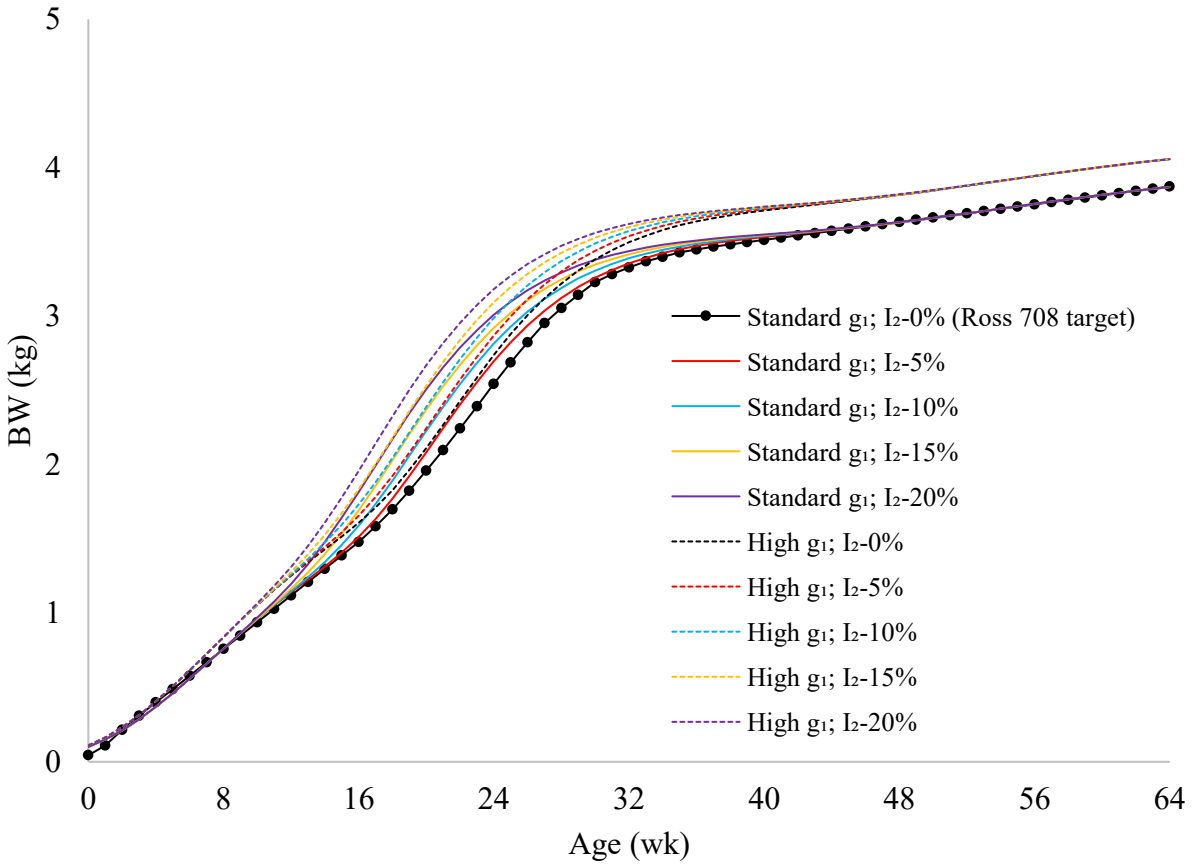

Figure 4. 1. Growth trajectories designed using estimated coefficients of a 3 phase Gompertz model. General model form was $BW_t = \sum_{i=1}^{i=3} g_i exp^{-exp^{-b_i(t-I_i)}}$ where BW_t was BW (kg) at time t (wk); g_i was the total amount of gain (kg) in phase i ; b_i was the growth rate coefficient; t was age (wk); I_i was the inflection point (wk), or the age at which growth for phase i reached its maximum rate. g_1 coefficient (g_1) was the prepubertal phase gain coefficient estimated by fitting the model to the standard Ross 708 recommended BW gain target (Standard g_1) or 10% higher (High g_1). Pubertal phase inflection point coefficient (I_2) was advanced by 5, 10, 15, and 20% creating inflection points at 21.16, 20.05, 18.94, and 17.82 wk of age, respectively.

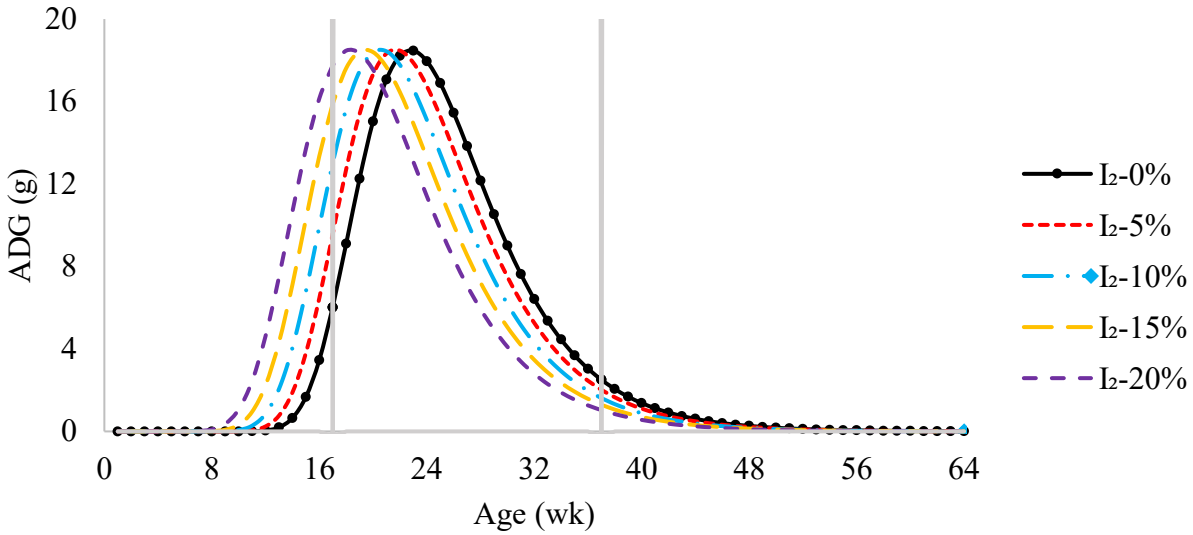

Figure 4. 2. Pubertal BW gain estimated by fitting a 3-phase Gompertz growth model to target BW of female Ross 708 broiler breeders. Standard pubertal inflection point (I_2) was advanced by 0, 5, 10, 15, or 20% creating inflection points at 22.29, 21.16, 20.05, 18.94, and 17.82 wk of age. Vertical grey reference lines show the timeframe (17 to 37 wk) for accumulation of 90% of the pubertal gain for the standard I_2. ADG was average daily gain.

5.0 Chapter 5: Architecture of Broiler Breeder Energy Partitioning Models

5.1 Abstract

A robust model that estimates the ME intake over broiler breeder lifetime is essential for formulating diets with optimum nutrient levels. The experiment was conducted as a randomized controlled trial with 40 Ross 708 broiler breeder pullets reared on one of ten target growth trajectories, which were designed with 2 levels of the amount of prepubertal growth and 5 levels of timing of growth around puberty. This study investigated the effect of growth pattern on energy efficiency of birds and tested the effects of chunking data into daily, 4-d, weekly, 2-wk, and 3-wk periods and the inclusion of random terms associated with individual maintenance ME and ADG requirements, and age on ME partitioning model fit and predictive performance. Model [I] was: $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d was daily ME intake (kcal/d); BW in kg; ADG_p was positive ADG; ADG_n was negative ADG (g/d); EM was egg mass (g/d); ε was the model residual. Models [II to IV] were nonlinear mixed models based on the model [I] with inclusion of a random term for individual maintenance requirement, age, and ADG, respectively. Model [II] – 3wk was chosen as the most parsimonious based on lower autocorrelation bias, closer fit of the estimates to the actual data (lower model MSE and closer R^2 to 1), and greater predictive performance among the models. Estimated ME partitioned to maintenance in model [II] – 3wk was 100.47 ± 7.43 kcal/$kg^{0.56}$, and the ME requirement for ADG_p, ADG_n, and EM were 3.49 ± 0.37; 3.16 ± 3.91; and 2.96 ± 0.13 kcal/g, respectively. Standard treatment had lower residual heat production (**RHP**; -0.68 kcal/kg $BW^{0.56}$) than high early growth treatment (0.79 kcal/kg $BW^{0.56}$), indicating greater efficiency in utilizing the ME consumed. Including random term associated with individual maintenance ME in a 3-wk chunk size provided a robust, biologically sound life-time energy partitioning model for breeders.

Key words: broiler breeder, feed restriction, energy partitioning model, prediction optimization, random term

5.2 Introduction

Creating robust energy intake models is important to formulate poultry diets with optimum levels of nutrients and to make economic decisions in the poultry industry. Metabolizable energy requirement models have been developed (Sakomura et al., 1993, 2003; Pishnamazi et al., 2015; Hadinia et al., 2018; Rabello et al., 2006; Reyes et al., 2012; Romero et al., 2009b; Sakomura, 2004; van der Klein et al., 2020) according to coefficients estimated for maintenance ME requirement per metabolic BW (kcal/$W^{0.75}$), daily body weight gain (kcal/g) and daily egg mass production (kcal/g). A valid estimation approach in these models should be able to estimate model coefficients with reasonable accuracy, lowest possible bias, and smallest variation. In statistics, the word "bias" refers to anything that causes the results to be incorrect in a systematic way. For example, if an analysis procedure ensures that the calculated results, on average, deviate from the true value, the results are said to be biased (Motulsky, 2010). The most challenging aspect of statistical analysis is making valid inferences, which indicates reaching general conclusions from limited data. As inference in mathematical modeling techniques is an important mechanism of information integration, inferential efficiency is the ability to incorporate additional information into the knowledge structure that can be used to focus the attention of the inference mechanisms in the most promising direction (Žebec et al., 2015). As an example, including random terms associated with different sources of unexplained variation in a modeling procedure can improve inferential efficiency. Every statistical inference is based on a list of assumptions (e.g., independency in a model residual), which need to be considered before interpreting the statistical results. Various statistical procedures need to be evaluated based on

their efficiency, which is a measure of quality and robustness of an estimator in a model. Essentially, a more efficient estimator needs fewer observations than a less efficient one to achieve a given performance. Thus, a robust procedure of creating energy partitioning models containing valid estimated coefficients for maintenance, growth, and egg production adds to existing studies in two ways. Firstly, it explicitly improves accuracy in modelling techniques, thereby going beyond the common mathematical perspective of modelling procedures. Secondly, it increases predictive performance of ME intake models, thereby matching nutrient supply with nutrient requirements of individual birds.

Dozza et al. (2013) developed a methodology to analyse naturalistic data from car driving studies. The volume and variety of the naturalistic data posed substantial challenges in robust data analysis. Although variety in data can be considered an advantage in modeling, the variety caused by unexplained sources of variation can influence precise calculation of model coefficients, leading to unreliability in using the models. The authors employed an analytical method called data chunking, which divided data into equivalent, elementary pieces of data before other data analysis steps. The purpose of this analytical method was to facilitate a robust and consistent calculation of parameters. Chunking data to different sizes was used to increase the robustness and sensitivity of parameter calculation by avoiding bias from data segments with heterogeneous durations.

Energy requirement predicting models have been used to establish optimized levels of dietary nutrients and more profitable feeding programs for poultry (Sakomura, 2004), yet the effect of chunking BW and production data to different sizes on the fitting and predictive performance of the models remains to be elucidated. We hypothesized that increasing data chunk size could account for unexplained variation in data caused by variation in health status and

voluntary activity level of birds, anomalies in real-time BW data recorded by a precision feeding (**PF**) system (You et al., 2021), and environmental conditions. Furthermore, the effect of including random terms associated with different model parameters (individual maintenance ME and age) on the fitting performance of the models has been investigated (van der Klein et al., 2020). It is not clear how inclusion of different random terms could bias the predictive performance of ME intake partitioning models.

ME intake lost as heat is equivalent to total heat production (**THP**) or ME for maintenance (**ME_m**) requirement of an animal (Zuidhof, 2019a). The ME_m requirement includes ingestion of feed, voluntary activity, immune response, and thermal regulation, which can be confounded by the individual variation and feed restriction level in broiler breeders (Zuidhof, 2019a). Residual feed intake (**RFI**) and residual heat production (**RHP**) are biological indicators of energetic efficiency of growth and egg production in poultry (Willems et al., 2013). Residual feed intake is defined as the difference between observed and predicted feed intake based on energy requirements for production and maintenance (Luiting, 1990; Kennedy et al. 1993). Residual heat production or residual maintenance ME requirement (**RME_m**) is the residual of the linear relationship between ME_m and ME intake (Romero et al., 2009a). The effects of increasing the amount of early growth and earlier timing of growth around puberty on feeding motivation and reproductive performance in broiler breeders has been discussed elsewhere (Chapter 4 of the current thesis). In the current paper we evaluate the effect of growth pattern on energy efficiency in breeders.

The objectives of the current study were to 1) evaluate inclusion of random terms associated with individual ME_m, ADG, and age in a ME partitioning model on residual dependency, model fitting and predictive performance; 2) evaluate how including random terms

associated with individual maintenance ME, ADG, and age could bias the ME partitioning model; 3) evaluate the effect of chunking BW, ADG, and egg production data into different chunk sizes (daily, 4-d, weekly, 2-wk, or 3-wk) on fitting and predictive performance of ME partitioning model; and 4) determine the effect of an increased (10%) prepubertal BW gain and earlier pubertal phase growth on energy efficiency of broiler breeders.

5.3 Materials and Methods

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

5.3.1 Experimental Design

The experiment was conducted as a randomized controlled trial with Ross 708 broiler breeder pullets (n=40) reared on one of ten target growth trajectories. The growth trajectories were designed with 2 levels of the amount of prepubertal growth and 5 levels of timing of growth around puberty (Afrouziyeh et al., 2021). A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. Growth phases 1, 2, and 3 corresponded roughly to prepubertal, pubertal, and post-pubertal growth phases, respectively. The model included phase-specific BW gain and time of growth inflection coefficients. Body weight trajectories were designed with two levels of prepubertal phase gain (**g_1**) coefficient as a discrete variable and 5 levels of pubertal growth phase inflection point (**I_2**) coefficient as a continuous variable. The g_1 was estimated from the breeder-recommended standard BW gain (**Standard g_1**) target, or 10% higher (**High g_1**) in the prepubertal growth phase. The second (pubertal) growth phase inflection point (I_2) was advanced

by 0, 5, 10, 15, or 20% of the coefficient estimated from the breeder-recommended target BW. The BW trajectories were applied to each individual bird using a PF system, which collected BW and feed intake data for each individual bird. Therefore, each bird was an experimental unit.

5.3.2 Animals and Management

The experimental protocol was previously described in full detail elsewhere (Chapter 4 of the current thesis). Briefly, 40 Ross 708 broiler breeder pullets were housed in a single environmentally controlled room containing 2 PF stations, from hatch to 43 wk of age at a stocking density of 3.0 birds per m^2. The PF stations (Zuidhof et al., 2017, 2019b) were used to apply the growth trajectory treatments and to control individual feed intake to achieve and maintain the assigned target BW curves. At 14 d of age, each bird was equipped with a wing band containing a radio frequency identification (**RFID**) transponder to be individually recognized by the PF system. The PF system recorded individual BW and individual feed intake throughout the experiment. The birds were fed commercial diets: starter (crumble; ME 2,726 kcal/kg, 21.0% CP, 1.00% Ca, and 0.70% available P) from hatch to d 34; grower (mash; ME 2,799 kcal/kg, 15.0% CP, 0.79% Ca, and 0.45% available P) from d 35 to d 179; and laying diet (crumble; ME 2,798 kcal/kg, 15.3% CP, 3.30% Ca, and 0.64% available P) from d 180 onward. Water was provided ad libitum throughout the experiment. The photoschedule was 24L:0D (100 lx) from d 0 to 3 then reduced to 8L:16D (15 lx) on d 4. Pullets were photostimulated at wk 22 as the photoperiod was increased to 11L:13D (20 lx). The photoperiod increased further to 12L:12D (25 lx) at wk 23, then again at wk 24 to 13L:11D (50 lx) for the remainder of the experiment. A trap-nest with 8 nesting sites and a nest box with 8 nesting sites equipped with RFID readers which identified and weighed eggs of individual hens were installed in the room at 14 wk of age; thus, the pullets had the chance to adapt to the nesting system prior to the onset of lay.

5.3.3 Data Collection

Individual BW and feed consumption data were collected by the PF system database. Observed ME intake was calculated by multiplying the observed daily feed intake (g) by the calculated dietary ME content (kcal/g). Eggs were collected from nest boxes, weighed, and assigned to individual birds daily.

5.3.4 Chunking Data

Chunking (Dozza et al., 2013) was implemented on data extracted from the PF system database to obtain means for chunks of daily, 4-d, weekly, 2-, or 3-week durations. Individual BW, BW gain, feed intake, ME intake, and egg mass (**EM**) were calculated for each chunk. Metabolizable energy intake models were developed for each chunk of data based on the chunk-specific calculated parameters involved in the models.

5.3.5 Metabolizable Energy Partitioning Models

One fixed effect model and 3 mixed effect models were evaluated in each chunk size of data (Table 5.1). Model [I] was the basic nonlinear model of ME intake as a function of metabolic BW, ADG, and EM production (based on Romero et al., 2009a). The metabolic BW scaling exponent was allowed to fluctuate in all models. The ADG values were divided into separate positive gain (ADG_p) and negative gain (ADG_n) variables. Models [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of random terms for individual maintenance ME, age, and ADG, respectively. Model [II] included a random term $u \sim N(0,V_u)$ associated with the coefficient of metabolic BW to separate individual variation in maintenance ME into between- and within-individual components. Model [III] included a random term $uu \sim N(0,V_{uu})$ associated with the coefficient of metabolic BW by different time

periods corresponding to chunk duration (daily, 4-d, weekly, 2-, or 3-wk durations) to separate age variation in maintenance ME into between- and within-individual components. Model [IV] included a random term $v \sim N(0, V_v)$ associated with the coefficient of ADG to separate individual variation in ADG into between- and within-individual components.

5.3.6 Test for Dependent Residuals

Autocorrelation in a model residual indicates a violation of the assumption of independence that is relied upon by many analyses (Dormann et al., 2007). Autocorrelation analysis was used to determine the extent to which chunking affected dependent residuals in the ME partitioning models. This analysis was used to estimate dependency across chunks and to determine the extent to which traditional statistical analysis (which requires independence of observations) was still possible to apply after chunking. Autocorrelation coefficient (**ACF**), coefficient of determination ($\mathbf{R^2}$) of residuals versus lag-residuals in the ME partitioning models, and Durbin Watson (**DW**) statistic were used to evaluate dependent residuals in the models:

$$DW = \frac{\sum_{i=1}^{n}(e_i - e_{i-1})^2}{\sum_{i=1}^{n} e_i^2}$$

where e_i was the residual for the i^{th} observation, e_{i-1} was the lagged residual for the $i\text{-}1^{th}$ observation, and n was the number of observations. In the current study, tabulated lower (d_L) and upper (d_U) critical values and were 1.285 and 1.721, respectively (n=40, α=0.05). The DW value was compared to the lower and upper critical values, d_L and d_U. If DW was lower than d_L, there was a positive autocorrelation (DW close to 0) in the error terms. If the calculated DW was higher than d_U, there was not an autocorrelation (DW close to 2) or there was a negative autocorrelation (DW close to 4) in the error terms. If DW was between d_L and d_U, the test was inconclusive (Cetin et al., 2007).

The notation of ACF (m=number of time periods between points) is the correlation between points separated by m time periods. Autocorrelation coefficient determines how correlated points are with each other, based on how many time steps by which they are separated.

$$ACF(m) = \frac{\sum_{t=k+m}^{n}(y_t - \bar{y})(y_{t-k} - \bar{y})}{\sum_{t=1}^{n}(y_t - \bar{y})^2}$$

where y_t was the residual at time t, $\bar{y}$ was the mean value for residual, y_{t-k} was the residual at the time before time t. Essentially, autocorrelation is a measure of the degree of correlation between past and future data points, for different degrees of time separation.

5.3.7 Model Comparison

In addition to the SD of the residuals, which was directly estimated in the NLMIXED procedure of SAS software (Version 9.4, SAS Institute Inc., Cary, NC), models were evaluated using model fitting and predictive performance criteria. Mean square error (**MSE**) and R^2 of the models were used to evaluate fitting performance of the models. Model fitting evaluation criteria were computed as follows:

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$$

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

where y_i was the i^{th} ME intake observation, $\hat{y}_i$ was the predicted value for the i^{th} ME intake observation, $\bar{y}_i$ was the mean value of ME intake, and n was the number of observations.

A K-fold cross validation method was used to evaluate the predictive performance of the models. The dataset was randomly partitioned into 5 (K = 5) mutually exclusive equal subsets and this procedure was repeated 10 times. Each time, K-1 subsets were used as a training set and one subset was used for testing. The R^2 of the relationship between observed and predicted ME

intake; the mean absolute error (**MAE**), MSE, and the root mean square error (**RMSE**) were calculated as cross validation statistics for the testing data (Yang and Huang, 2014). Cross validation statistics were computed as follows:

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|y_i - \hat{y}_i|$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$

Where y_i was the ith ME intake observation, $\hat{y}_i$ was the predicted value for the ith ME intake observation, and n was the number of observations.

5.3.8 Evaluation of Energy Efficiency

Total heat production, RHP, and RFI were evaluated using the model of choice (model [II] – 3wk) and used as indicators of energy efficiency of growth and egg production. Total heat production was calculated as daily maintenance requirement ((a + u) × $BW^{0.56}$) and reported as kcal/d. The residual of the linear relationship between bird ME_m (kcal/$BW^{0.56}$/d) and ME intake (kcal/$BW^{0.56}$/d) was calculated as RHP (kcal/kg $BW^{0.56}$). The slope of the recent relationship represented the proportion of ME lost as heat per unit of ME intake. Predicted ME intake was estimated using the model [II] – 3wk. Residual feed intake was calculated as the difference between observed and predicted ME intake.

5.3.9 Statistical Analysis

All statistical analyses were performed using SAS software (Version 9.4, SAS Institute Inc., Cary, NC). Metabolizable energy partitioning models were fitted using the NLMIXED procedure. Partitioning of dataset into training and testing subsets, for cross validation method,

was performed using the SURVEYSELECT procedure. The linear regression between maintenance requirement coefficient and ME intake was conducted using the MIXED procedure. Analysis of covariance was conducted on ME_m, RHP, and RFI using the HPMIXED and MIXED procedures, with g_1 and time period as discrete sources of variation, and I_2 as a continuous predictor variable. Period was included in the model as a random effect with individual birds as the subject to account for within-bird variation. Pairwise differences between means within each period were determined using Tukey's HSD test and were reported as different when $P \leq 0.05$.

5.4 Results and Discussion

5.4.1 Estimated Coefficients in ME Intake Models

Increasing chunk size from daily to 3-wk period decreased coefficient for metabolic BW (maintenance requirement) and instead increased coefficients for daily gain and EM in all models (Tables 5.2, 5.3, 5.4, 5.5, and 5.6). For instance, for a 2.00 kg bird, maintenance energy requirement (kcal/d) ranged from 147.10 to 216.33 kcal/d for the 3-wk (model [IV] – 3wk) and daily (model [III] – daily) chunk sizes, respectively. These were similar to previously reported estimates for ME_m requirement, which ranged from 147.6 to 245.2 kcal/d for a 2.00 kg broiler breeder pullet or hen (Sakomura et al., 2003; Romero et al., 2011; Hadinia et al., 2018; van der Klein et al., 2020). An estimated ME_m for a 2.00 kg broiler breeder hen in the current study was 148.1 kcal/d ($119.73 \times 2.00^{0.49}$; based on the coefficients of model [II] – 3wk Table 5.6), which is less than that reported by van der Klein et al. (2020), in which weekly chunked data were used ($130.64 \times 2.00^{0.58} = 195.1$ kcal/d; based on the coefficients in the model of choice in their study). This wide range for ME_m requirement in the literature was due to animal behavior, bird age, strain, temperature, and dietary energy level. Furthermore, different housing systems (20%

greater ME_m requirement in floor pens than cage-raised ones; Rabello et al., 2006), feed intake (10% greater heat increment in *ad libitum* fed birds compared to that of pullets restricted to 54% of the *ad libitum* feed intake; Sakomura et al., 2003), and methodology being used to estimate ME_m requirement (indirect calorimetry, Spratt et al., 1990; comparative slaughter method, Rabello et al., 2006; Reyes et al., 2012; mathematical modelling approach, van der Klein et al., 2020) could affect the estimated ME_m requirement. This study revealed that chunk size of data used in modelling of ME partitioning can also affect the estimated ME_m requirement.

The coefficient for ADG_p, which indicated ME requirement for each gram of gain, ranged from 0.46 to 3.66 kcal/g for the daily (model [III] – daily) and 3-wk (model [III] – 3wk) chunks, respectively. A wide range of ME requirements for gain has been reported from 0.71 to 5.80 kcal/g in the literature (Sakomura, 2004; Reyes et al., 2012, Hadinia et al., 2018). Variation in ME requirements for growth can be associated with differences in composition of gain as affected by stage of maturity; fat tissue contains a higher energy content (9.1 kcal/g) compared to lean tissue (5.5 kcal/g of DM basis or 3.7 kcal/g of wet tissue; Leeson and Summers, 2001). As age increases, the amount of body fat increases (Leenstra, 1986). Lean mass increases until egg peak production and then there is a loss in lean tissue towards 50 wk of age in broiler breeders; this process is a net mobilization of lean tissue to support egg production (Salas et al., 2010; van Emous et al., 2015; Vignale et al., 2016). However, fat reserves increase throughout the egg production phase and reaches a maximum at 50 wk of age (van Emous et al., 2015; Caldas et al., 2019). Thus, the ME requirement for gain should increase as BW increases with age, with a fast accumulation rate for energetically expensive fat mass towards 50 wk of age.

The ME requirements for each gram of EM ranged from 1.60 kcal/g with the daily chunk size (model [III] – daily) to 2.97 kcal/g with the 2 wk chunk size (model [II] – 2wk). The ME

requirement for egg production ranges from 1.90 to 3.15 kcal/g (Sakomura, 2004; Romero et al., 2009b; Reyes et al., 2012; van der Klein et al., 2020). The energy content of broiler breeder eggs ranges from 1.33 kcal/g (Sibbald, 1979) to 1.79 kcal (Chwalibog, 1992) with an average value of 1.54 kcal/g (Sakomura, 2004). With an average efficiency of ME utilization for energy deposition in broiler breeder eggs (64%), an expected ME requirement for egg production would be around 2.40 kcal/g (Sakomura, 2004).

Variation of ADG_p, ADG_n, and EM decreased as the chunk size increased (data not shown). Reduced variation of an independent variable could be due to sampling choices, which subsequently could be a source of variation in estimated coefficients in a ME partitioning model. Furthermore, stability of estimated regression coefficients in a model is associated with the variance of the independent variable and sample size (O'Brien, 2007). Thus, it can be hypothesized that a reduction in the variation of the ADG and EM due to an increased chunk size was a possible reason for an increase in their estimated coefficients. This hypothesis can be accepted by comparing the pattern of ME requirement for egg production in the literature and the current study. The ME requirement for egg production has been reported as 1.78 kcal/g in a semi-weekly chunked data (Pishnamazi et al., 2015); 2.10 kcal/g in a semi-weekly chunked data until 32 wk of age and weekly chunked data thereafter (Romero et al., 2009b); 2.40 and 2.42 kcal/g in a weekly chunked data (Reyes et al., 2011; van der Klein et al., 2020); and 2.96 kcal/g in a 3-wk chunked data (current study). Therefore, it can be concluded that using a longer chunk size (3-wk vs semi-weekly or weekly) in calculating the average value of individual BW and feed intake to establish a ME intake partitioning model can highlight the contribution of ADG and EM in the model by increasing their estimated coefficient. More specifically, longer chunk

might smooth out the day-to-day variation and associated costs of building up nutrients and deposition of nutrients in the egg in breeders which did not lay an egg every day.

5.4.2 Model Comparison

5.4.2.1 Effect of Chunk Size. Increasing chunk size of data decreased SD of residuals in each model (Table 5.2, 5.3, 5.4, 5.5, and 5.6). The SD of residuals decreased for 4-d, weekly, 2-wk, and 3-wk period chunk sizes compared to that of daily chunk size by 30.6, 37.5, 47.9, and 52% in model [I], 31.2, 38.1, 48.7, and 52.3% in model [II], 34.32, 41.1, 48.3, and 53.3% in model [III], and 32.2, 39.2, 47.2, and 51.3% in model [IV], respectively, which indicated that more variation was accounted for in 3-wk chunk size. The smaller the residual SD, the closer is the fit of the estimate to the actual data. Therefore, chunking data to 3-wk periods provided closest fit of the ME intake estimates to the actual ME intake, demonstrating more precise and more accurate (close to being correct) estimation of coefficients in the ME partitioning model. An analytical method is precise when repeated measurements give very similar results. van der Klein et al. (2020) raised a concern about an instability issue in estimated coefficients of a ME partitioning model containing a random term associated with the individual bird nested within a random term of age. The authors hypothesized that the model did not converge because of the large variability in age at first egg between birds as the birds were in different physiological states at the same age. They concluded that individual bird rather than age would explain a large proportion of the differences in ME_m requirements over age in their study. However, the results of the current study showed that other factors such as chunk size of data would affect stability and precision of estimated coefficients in a model.

Within each model, increasing chunk size of data increased fitting performance of ME partitioning models by reducing MSE and increasing R^2 of the fitted models (Table 5.7). It also

increased predictive performance of the models by reducing RMSE and R^2 of the linear relationship between observed and predicted ME intake in the testing subsets of a 5-fold cross validation. It is possible that increasing chunk size from daily to 3-wk reduced the influence of outliers caused by unaccounted sources of error such as environmental condition, voluntary activity level, and health status of the birds on the model parameters (Zuidhof, 2019a).

Increasing chunk size affected autocorrelation bias differently across the models (Figures 5.1 to 5.3). Chunking data to 3-wk periods resulted in the lowest autocorrelation bias in all models except for model [III] where the lowest ACF was calculated in daily chunk size (Figure 5.1). Lower autocorrelation bias was detected by lower ACF, lower R^2 of the relationship between residuals and lag-residuals (Figure 5.2), and a DW value closer to 2 (Figure 5.3).

5.4.2.2 Effect of Random Terms. The residual SD decreased for models [II], [III] and [IV] as compared to model [I] by 1.31, 11.44, and 1.44% in daily chunked, 2.13, 16.15, and 3.78% in 4-d chunked, 2.23, 16.63, and 4.09% in weekly chunked, 2.75, 11.99, and 0% in 2-wk chunked, and 2.04, 13.86, and 0% in 3-wk chunked data (Tables 5.2, 5.3, 5.4, 5.5, and 5.6). Incorporating a random term associated with individual ME_m requirement or age partitioned part of the residual SD (σ_e) into bird-specific (σ_u) and age-specific (σ_{uu}) variation in maintenance. Including random term associated with individual ADG reduced residual SD in all chunk sizes except for the 2-wk and 3-wk periods. In fact, model [IV] was identical to model [I] in the 2-wk and 3-wk periods. This might be because increasing chunk size beyond weekly period, had already reduced variation in ADG in a way that including the random term for ADG did not further reduce the residual SD. This can be confirmed by reduction in ADG variation with increasing chunk size which was discussed earlier in this paper.

5.4.2.3 Selection of the Model of Choice. Across all chunk sizes, including a random term associated with age (model [III]) resulted in the lowest MSE (Table 5.7). Among the models, model [III] – 3wk showed the lowest MSE and closet R^2 to 1 (best fitting performance) followed by the models [III] – 2wk and [II] – 3wk (Table 5.7). However, models [III] – 3wk and [III] – 2wk showed autocorrelation bias (Figures 5.1 to 5.3), which is a considerable disadvantage. As already discussed, a DW lower than the lower critical value (d_L = 1.285 in this study) indicated positive autocorrelation in the model residual. DW values of the models [III] – 3wk and [III] – 2wk were 0.910 and 0.977, respectively indicating positive autocorrelation in their residuals (Figure 5.2). Autocorrelation in the residual of a model indicates a violation of the assumption of independence that is relied upon by many analyses. Therefore, predictions of a model with high autocorrelation may be inefficient. This indicates that there was likely unexplained variation which, if accounted for, would improve inferential efficiency. Thus, residuals independency assumption was prioritized over the model fitting performance by selecting a model with a lower autocorrelation bias in the first place and greater fitting performance in the second place. Model [III] – daily with a DW value of 1.634 showed the lowest autocorrelation bias in the residual followed by model [II] – 3wk with a DW value of 1.561, which both fell between the lower and upper critical DW (d_L = 1.285 and d_U = 1.721). However, model [III] – daily was not a reliable model from either fitting or predictive perspectives (Table 5.7). Model [III] – daily did not meet the requirements of the best fitting (i.e., lower MSE and an R^2 closer to 1) nor the predictive performance criteria (i.e., lower MSE, RMSE, and MAE and an R^2 closer to 1 in the testing models in a K-fold cross validation). Based on the above-mentioned information, model [II] – 3wk, with a reliable fitting and predictive

performance, was selected as the model of choice for further discussion of ME_m requirements and energy efficiency evaluation in this study.

Including bird-specific random terms associated with individual maintenance ME or ADG requirements reduced autocorrelation bias compared to the fixed effect models in all chunk sizes. However, including the random term associated with age increased autocorrelation bias compared to the fixed effect model except in the daily chunk size. Thus, it can be hypothesized that including a random term associated with age can bias the model residual independency assumption except if the data is used in the daily chunk size. This was because by increasing chunk size (duration of periods) the number of periods as a proxy of "age" decreased, and as discussed earlier the variation of data decreased; as a result, dependency in the model residual would increase. Model [III] – daily showed the lowest dependent residual as including a random term associated with age in the model where it had maximum number of time periods (daily) accounted for the variation due to the age effect. For all chunk sizes, including a random term associated with individual maintenance ME requirement biased the predictive performance of the models compared to the scenario where the random term was associated with age.

5.4.3 Energy Efficiency

Earlier pubertal growth increased ME_m in birds, which was greater in High g_1 birds (2.12 kcal/d/wk of earlier pubertal growth) than in Standard g_1 birds (1.50 kcal/d/wk of earlier pubertal growth; $P < 0.001$, Table 5.8). Factors contribute to energy loss as heat production have been categorized into dietary factors such as nutrient composition and feed form (Lopez and Leeson, 2008), environmental factors such as temperature (Pishnamazi et al., 2015; Rabello et al., 2006), and animal factors including age, sex, genetic potential, feed intake (Swennen et al., 2004), reproductive status (van der Klein et al., 2020), health status (van Eerden et al., 2006), and

activity level (van Milgen et al., 2001). In the current study, some animal factors such as feed intake and activity level could have contributed to the increase in ME_m requirement of birds with earlier pubertal growth. Earlier pubertal growth increased BW, frequency of daily station visits (as an indicator of activity level), feed intake, and subsequently feed intake-associated (diet-induced) thermogenesis in broiler breeders, which consequently required more energy for maintenance (Chapter 4 of the current thesis).

The linear relationship between average daily ME intake and ME_m for the total experimental period (Figure 5.4) has two main applications: first, it measures bias in the random term, which may be explained by changes in ME_m expenditure at various levels of ME intake. Second, the slope coefficient represents the proportion of increased ME intake that is used for ME_m requirement (lost as heat), which is the heat increment of feeding. In the current study, the model [II] – 3wk predicted that ME_m (kcal/$BW^{0.56}$) increased by 0.013 kcal/kcal of ME intake; in other words, 1.3% of the increase in ME intake was used for ME_m requirement and lost as heat from 2 to 43 wk of age. In the literature estimated heat increment of feeding has been reported as 52% during the life-time of broiler breeders from 2 to 55 wk of age, 79% during the rearing phase from 2 to 20 wk of age, 44% during the laying phase from 22 to 55 wk of age (van der Klein et al., 2020), 19 and 34% during the laying phase from 20 to 60 wk of age (Romero et al., 2009a,b), and 87% from 10 to 23 wk of age (Hadinia et al., 2018). Animal factors such as bird age, composition of gain, and reproductive status (van der Klein et al., 2020) and dietary factors such as diet composition (Romero et al., 2009a) can affect heat increment of feeding. The lower coefficient for the slope of ME_m and ME intake relationship in the current study (1.3%) compared to that of in the literature (19 to 87%) indicated a lower bias in the model [II] – 3wk, which has accounted for unexplained feed intake-associated heat production. The vertical

distance between each individual point and the regression line (Figure 5.4) corresponded to the RHP value. The Standard g_1 treatment birds had a lower RHP than that of their counterparts in the High g_1 treatment. Figure 5.4 shows that most of the individuals in the Standard g1 treatment had a RHP lower than the regression line representing a negative value for the RHP. Thus, Standard g_1 birds were more efficient in utilizing dietary energy compared to the High g_1 birds.

For every week of earlier pubertal growth, RHP increased by 0.20 and 0.48 kcal/kg $BW^{0.56}$ for the Standard and High g_1 birds ($P = 0.005$, Table 5.8). Standard g_1 birds had lower RHP than that of the High g_1 birds (-0.68 ± 0.1 vs. 0.79 ± 0.11 kcal/kg $BW^{0.56}$, $P < 0.001$). It could be hypothesized that a higher degree of feed restriction in the Standard g_1 birds compared with that of the High g_1 birds provided stimulus for a metabolic shift in the Standard g_1 birds to become more energetically conservative with ME partitioning to HP. This means that the Standard g_1 birds were more energetically efficient in utilizing the ME intake compared to their counterparts in the High g_1 treatment. This was expected as feed restriction may increase efficiency by reducing heart rate, blood pressure, and body temperature in restricted fed birds (Savory et al., 2006). Furthermore, both caloric restriction and low RFI induced a shift to an energetically conservative mode in rodents (Selman et al., 2006) and pigs (Lkhagvadorj et al., 2010) by downregulating steroidogenesis and lipogenesis in both liver and adipose tissue.

Increasing prepubertal phase BW gain increased RFI (-1.22 ± 1.22 kcal/d in the Standard g_1 vs. 2.12 ± 1.30 kcal/d in the High g_1 treatment, $P = 0.011$, Table 5.8), which was in line with the RHP results. For each week of earlier pubertal growth RFI increased by 1.72 kcal/d ($P = 0.006$, Table 5.8). It has been previously shown that RFI values can be confounded by heat increment of feeding (Swennen et al., 2007). However, RHP is an indicator of energy efficiency which is not confounded by feed intake, heat increment of feeding, BW gain, and egg production

(Romero et al., 2009a). Thus, RHP can be used as a better estimator for energy efficiency for maintenance requirements compared to RFI.

5.4.4 Comparison of Current Study Model with Other ME Intake Models

Model [II] – 3wk overestimated ME requirement from 2 to 30 wk, 2 to 18 wk, and 2 to 6 wk of age and underestimated it from 31 to 43 wk, 19 to 43 wk, and 3 to 43 wk of age compared to the models developed by van der Klein et al. (2020), Pishnamazi et al. (2015), and Reyes et al. (2012), respectively (Figure 5.5). The difference between the estimated ME requirement values in the current study and those of Reyes et al. (2012) could be at least partially explained by the different genetic strain used in these studies. Reyes et al. (2012) used Cobb 500, which have heavier BW compared to Ross 708 at the same age (2,600 g vs 2,245 g at 22 wk of age; Cobb 500, 2019; Aviagen, 2016). Although Pishnamazi et al. (2015) and van der Klein et al. (2020) used the same strain as the current study (Ross 708), different chunk size of the data (weekly) was used in their studies to build the ME intake models compared to the model of choice in the current study (3-wk chunk size). The energy requirement estimated by model [II] – 3wk was higher than the Ross 708 guideline (Aviagen, 2016) from 2 to 12 wk of age but lower than that from 13 to 43 wk of age; possibly the overestimation from 2 to 12 wk of age was due to using a higher BW profile (on average) compared to the guideline. If that is the case, that might have increased our prediction for the ME_m requirement. Overall, the previously published models with Ross 708 strain (Pishnamazi et al., 2015; van der Klein et al., 2020) along with the model developed by the current study estimated a lower energy requirement during the lifetime or after 12 wk of age compared to the recommended age-specific ME intake data by Ross 708 guideline (Figure 5.5). Comparison of estimated energy requirement by three studies revealed that the breeder recommended ME intake does not likely match the guideline-recommended target BW.

It means that by applying guideline ME intake recommendation a higher achieved BW would be expected (Figure 5.6).

5.5 Conclusions

To increase robustness of broiler breeder energy partitioning models, a novel chunking procedure was applied on precision feeding system data. To our knowledge, this is the first investigation of the effects of chunking approach on the ME partitioning models bias, fitting, and predictive performance. Increasing chunk size of data provided closer fit of the models estimated coefficients to the actual data by accounting for more variation in the residuals. Using a 3-wk chunk size provided a model with lower bias, smallest variation, and greater accuracy and precision in estimated coefficients. A mixed effect ME partitioning model containing a random term associated with individual maintenance requirement in a 3-wk chunked data (model [II] – 3wk) increased inferential efficiency. The model can be used as a tool to estimate ME requirements and to facilitate choosing a precise energy level in feed formulation practices. Furthermore, applying Ross 708 guideline data in the model suggested a revision on the breeder-recommended target BW. The current study results indicated that an earlier pubertal growth strategy could reduce energy efficiency in broiler breeders.

5.6 Acknowledgment

Funding from Alberta Agriculture and Forestry (Edmonton, AB), Poultry Innovation Partnership (Edmonton, AB), scholarship support of Alberta Innovates and Alberta Advanced Education, technical assistance from Chris Ouellette and in-kind support for the precision feeding system from Xanantec Technologies, Inc. (Edmonton, AB, Canada) is gratefully acknowledged.

5.7 References

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5.8 Tables

Table 5. 1. Functional specifications of the evaluated models.

Model[1]	Function specification
I	$MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$
II	$MEI_d = (a + u) \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$
III	$MEI_d = (a + uu) \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$
IV	$MEI_d = a \times BW^b + (c + v) \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$

[1]Estimated coefficients are lowercase letters. MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); u = bird-specific random term associated with individual maintenance; uu = age related random term; v = bird-specific random term associated with individual ADG; ε = residual error. Model [I] was a fixed effect model. Model [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for individual maintenance ME, age, and ADG, respectively.

Table 5. 2. Regression coefficients of nonlinear ME intake models analysed based on daily data, representing ME partitioning to maintenance, gain, and egg production in Ross 708 broiler breeder females.

Model[1]	Model [I]			Model [II]			Model [III]			Model [IV]		
Coefficient[2]	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|
a (kcal/BW^b)	152.04	1.53	< 0.001	153.58	1.88	< 0.001	157.27	2.19	< 0.001	152.04	1.54	< 0.001
b	0.46	0.01	< 0.001	0.45	0.01	< 0.001	0.46	0.01	< 0.001	0.46	0.01	< 0.001
c (kcal/g)	0.56	0.06	< 0.001	0.53	0.06	< 0.001	0.46	0.05	< 0.001	0.58	0.11	< 0.001
d (kcal/g)	0.58	0.08	< 0.001	0.57	0.08	< 0.001	0.63	0.08	< 0.001	0.58	0.08	< 0.001
e (kcal/g)	1.86	0.03	< 0.001	1.87	0.03	< 0.001	1.60	0.03	< 0.001	1.88	0.02	< 0.001
σ_u				6.56	0.89	< 0.001						
σ_{uu}							21.92	1.07	< 0.001			
σ_v										0.57	0.07	< 0.001
ε	60.13	0.42	< 0.001	59.34	0.42	< 0.001	53.25	0.38	< 0.001	59.26	0.42	< 0.001

[1]Model [I] was a fixed effect model with the form of $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d). Model [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for individual maintenance ME, age, and ADG, respectively. u = bird-specific random term associated with individual maintenance; uu = age related random term; v = bird-specific random term associated with individual ADG.

Table 5. 3. Regression coefficients of nonlinear ME intake models analysed based on a 4-d data, representing ME partitioning to maintenance, gain, and egg production in Ross 708 broiler breeder females.

Model[1]	Model [I]			Model [II]			Model [III]			Model [IV]		
Coefficient[2]	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|
a (kcal/BW^b)	125.76	3.22	< 0.001	128.42	3.29	< 0.001	133.75	4.17	< 0.001	124.1	3.25	< 0.001
b	0.51	0.01	< 0.001	0.49	0.01	< 0.001	0.55	0.02	< 0.001	0.51	0.01	< 0.001
c (kcal/g)	2.03	0.15	< 0.001	1.94	0.15	< 0.001	1.69	0.15	< 0.001	2.14	0.20	< 0.001
d (kcal/g)	0.86	0.37	0.024	0.83	0.37	0.033	1.28	0.32	0.023	0.81	0.36	0.035
e (kcal/g)	2.36	0.06	< 0.001	2.36	0.05	< 0.001	1.81	0.07	< 0.001	2.41	0.05	< 0.001
σ_u				5.52	0.88	< 0.001						
σ_{uu}							19.75	1.93	< 0.001			
σ_v										0.74	0.10	< 0.001
ε	41.71	0.59	< 0.001	40.82	0.58	< 0.001	-34.97	0.50	< 0.001	40.13	0.57	< 0.001

[1]Model [I] was a fixed effect model with the form of $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d). Model [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for individual maintenance ME, age, and ADG, respectively. u = bird-specific random term associated with individual maintenance; uu = age related random term; v = bird-specific random term associated with individual ADG.

Table 5. 4. Regression coefficients of nonlinear ME intake models analysed based on weekly data, representing ME partitioning to maintenance, gain, and egg production in Ross 708 broiler breeder females.

Model[1]	Model [I]			Model [II]			Model [III]			Model [IV]		
Coefficient[2]	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|
a (kcal/BW^b)	116.48	4.45	< 0.001	119.73	4.436	< 0.001	119.76	5.79	< 0.001	118.34	4.28	< 0.001
b	0.52	0.02	< 0.001	0.49	0.02	< 0.001	0.60	0.04	< 0.001	0.50	0.02	< 0.001
c (kcal/g)	2.58	0.21	< 0.001	2.45	0.21	< 0.001	2.44	0.24	< 0.001	2.50	0.24	< 0.001
d (kcal/g)	0.65	0.48	0.17	0.72	0.47	0.13	0.94	0.41	0.023	0.70	0.46	0.14
e (kcal/g)	2.66	0.08	< 0.001	2.66	0.07	< 0.001	1.98	0.10	< 0.001	2.69	0.07	< 0.001
σ_u				5.13	0.95	< 0.001						
σ_{uu}							19.16	2.52	< 0.001			
σ_v										0.73	0.11	< 0.001
ε	37.57	0.70	< 0.001	36.73	0.69	< 0.001	-31.32	0.59	< 0.001	36.03	0.68	< 0.001

[1]Model [I] was a fixed effect model with the form of $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d). Model [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for individual maintenance ME, age, and ADG, respectively. u = bird-specific random term associated with individual maintenance; uu = age related random term; v = bird-specific random term associated with individual ADG.

Table 5. 5. Regression coefficients of nonlinear ME intake models analysed based on a 2-wk data, representing ME partitioning to maintenance, gain, and egg production in Ross 708 broiler breeder females.

Model[1]	Model [I]			Model [II]			Model [III]			Model [IV]		
Coefficient[2]	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|
a (kcal/BW^b)	106.54	5.70	< 0.001	109.51	5.61	< 0.001	113.14	7.58	< 0.001	106.54	5.70	< 0.001
b	0.53	0.03	< 0.001	0.51	0.03	< 0.001	0.59	0.05	< 0.001	0.53	0.03	< 0.001
c (kcal/g)	3.14	0.28	< 0.001	3.02	0.28	< 0.001	2.81	0.34	< 0.001	3.14	0.28	< 0.001
d (kcal/g)	3.57	2.24	0.11	3.77	2.23	0.091	3.38	2.01	0.10	3.57	2.24	0.12
e (kcal/g)	2.96	0.10	< 0.001	2.97	0.10	< 0.001	2.33	0.15	< 0.001	2.96	0.10	< 0.001
σ_u				4.63	1.05	0.001						
σ_{uu}							15.30	2.95	< 0.001			
σ_v										1.05	0.05	< 0.001
ε	31.27	0.81	< 0.001	30.41	0.81	< 0.001	-27.52	0.73	< 0.001	31.27	0.81	< 0.001

[1]Model [I] was a fixed effect model with the form of $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d). Model [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for individual maintenance ME, age, and ADG, respectively. u = bird-specific random term associated with individual maintenance; uu = age related random term; v = bird-specific random term associated with individual ADG.

Table 5. 6. Regression coefficients of nonlinear ME intake models analysed based on a 3-wk data, representing ME partitioning to maintenance, gain, and egg production in Ross 708 broiler breeder females.

Model [1]	Model [I]			Model [II]			Model [III]			Model [IV]		
Coefficient[2]	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|	Estimate	SEM	Pr > |t|
a (kcal/BW^b)	97.91	7.52	< 0.001	100.47	7.43	< 0.001	98.94	9.34	< 0.001	97.91	7.52	< 0.001
b	0.58	0.04	< 0.001	0.56	0.04	< 0.001	0.65	0.07	< 0.001	0.58	0.04	< 0.001
c (kcal/g)	3.59	0.38	< 0.001	3.49	0.37	< 0.001	3.66	0.46	< 0.001	3.59	0.38	< 0.001
d (kcal/g)	2.60	3.90	0.50	3.16	3.91	0.42	2.78	3.40	0.42	2.60	3.90	0.50
e (kcal/g)	2.96	0.13	< 0.001	2.96	0.13	< 0.001	2.47	0.18	< 0.001	2.96	0.13	< 0.001
σ_u				3.45	1.18	0.011						
σ_{uu}							12.53	2.98	< 0.001			
σ_v										1.02	0.03	< 0.001
ε	28.85	0.92	< 0.001	28.26	0.93	< 0.001	24.85	0.81	< 0.001	28.85	0.92	< 0.001

[1]Model [I] was a fixed effect model with the form of $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d). Model [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for individual maintenance ME, age, and ADG, respectively. u = bird-specific random term associated with individual maintenance; uu = age related random term; v = bird-specific random term associated with individual ADG.

Table 5. 7. Model fitting and performance statistics of nonlinear ME intake models analysed based on daily, 4-d, weekly, 2-wk, and 3-wk chunked data, representing ME partitioning to maintenance, gain, and egg production in Ross 708 broiler breeder females.

	Model fitting statistics[2]		Cross validation statistics[3]			
Model [1]	MSE	R^2	MAE	MSE	RMSE	R^2
[I] – daily	3616	0.730	42.8	3689	60.7	0.725
[II] – daily	3510	0.738	42.0	3573	59.7	0.734
[III] – daily	2762	0.794	37.8	2774	52.8	0.791
[IV] – daily	3501	0.739	42.2	3563	59.6	0.734
[I] – 4d	1739	0.845	28.4	1726	41.5	0.847
[II] – 4d	1649	0.853	27.7	1635	40.3	0.855
[III] – 4d	1190	0.894	23.0	1161	34.2	0.895
[IV] – 4d	1592	0.859	27.7	1566	39.4	0.862
[I] – weekly	1412	0.872	25.2	1382	37.1	0.875
[II] – weekly	1327	0.880	24.6	1305	36.1	0.882
[III] – weekly	954	0.914	20.4	937	30.7	0.915
[IV] – weekly	1273	0.885	24.6	1259	35.5	0.886
[I] – 2wk	978	0.908	21.5	1047	32.3	0.903
[II] – 2wk	900	0.915	20.7	974	31.0	0.911
[III] – 2wk	737	0.931	18.4	776	27.8	0.928
[IV] – 2wk	978	0.908	20.7	919	30.1	0.916
[I] – 3wk	832	0.918	20.4	875	29.6	0.914
[II] – 3wk	778	0.923	19.0	797	27.9	0.923
[III] – 3wk	601	0.941	16.6	612	24.7	0.939
[IV] – 3wk	832	0.918	19.8	786	27.8	0.924

[1]Model [I] was a fixed effect model with the form of $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d). Model [II], [III], and [IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for individual maintenance ME, age, and ADG, respectively. The data was chunked to daily, 4-d, weekly, 2-wk, and 3-wk sizes.

[2]MSE: Mean squared error; R^2: Coefficient of determination of observed ME intake with predicted ME intake by the models.

[3]MAE: Mean absolute error; RMSE: Root mean square error; R^2: Coefficient of determination of observed ME intake with predicted ME intake by the testing model in a k-fold cross validation.

Table 5. 8. Effects of pre-pubertal BW gain (g_1) and pubertal growth inflection (I_2) on maintenance energy requirement (ME_m), residual heat production[1] (RHP), and residual feed intake[2] (RFI) in Ross-708 broiler breeder females.

			Model [II] – 3wk [4]					
Effect[3]	g_1	I_2	ME_m	SEM	RHP	SEM	RFI	SEM
			kcal/d		kcal/kg $BW^{0.56}$		kcal/d	
g_1	Standard		157.2^{b}	0.25	-0.68^{b}	0.10	-1.22^{b}	1.22
	High		165.1^{a}	0.28	0.79^{a}	0.11	2.12^{a}	1.30
I_2		17.83	166.2^{a}	0.36	1.23^{a}	0.16	4.72^{a}	1.58
		18.95	161.9^{b}	0.42	-0.26^{bc}	0.18	0.13^{ab}	1.76
		20.06	161.3^{b}	0.39	0.16^{b}	0.17	-0.19^{ab}	1.67
		21.18	158.5^{c}	0.44	-0.30^{bc}	0.19	-0.20^{ab}	1.84
		22.29	157.8^{c}	0.36	-0.55^{c}	0.16	-2.20^{b}	1.58
$I_2 \times g_1$	Standard	17.83	160.9^{d}	0.50	-0.07^{cd}	0.22	3.69^{ab}	2.06
		18.95	159.3^{d}	0.58	-0.18^{cde}	0.26	-0.19^{ab}	2.33
		20.06	156.1^{e}	0.50	-1.16^{e}	0.22	-2.32^{ab}	2.06
		21.18	155.0^{e}	0.50	-1.22^{e}	0.22	-3.22^{b}	2.06
		22.29	154.7^{e}	0.50	-0.75^{de}	0.22	-4.06^{b}	2.06
	High	17.83	171.4^{a}	0.50	2.54^{a}	0.22	5.74^{a}	2.06
		18.95	164.6^{bc}	0.58	-0.34^{cde}	0.26	0.45^{ab}	2.33
		20.06	166.5^{b}	0.58	1.47^{ab}	0.26	1.93^{ab}	2.33
		21.18	162.0^{cd}	0.70	0.62^{bc}	0.31	2.82^{ab}	2.79
		22.29	161.0^{d}	0.50	-0.36^{cde}	0.22	-0.34^{ab}	2.06
I_2			-1.50	0.15	-0.20	0.06	-1.72	0.53
$I_2 \times g_1$	Standard		-1.50	0.15	-0.20	0.06	-1.72	0.53
	High		-2.12	0.36	-0.48	0.16	-1.04	1.30
Source of variation			*P*-value					
g_1			< 0.001		< 0.001		0.011	
I_2			< 0.001		0.002		0.006	
$I_2 \times g_1$			< 0.001		0.005		0.38	
period			< 0.001		0.061		< 0.001	

[1]Residual heat production (RHP) was the residual of the linear relationship between ME_m and ME intake.

[2]Residual feed intake (RFI) was defined as the difference between observed and predicted feed intake based on energy requirements for production and maintenance.

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

[4]Model [II] – 3wk was a mixed effect model with inclusion of a random term for individual maintenance ME. The model was $MEI_d = (a + u) \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); u = bird-specific random term associated with individual maintenance.

5.9 Figures

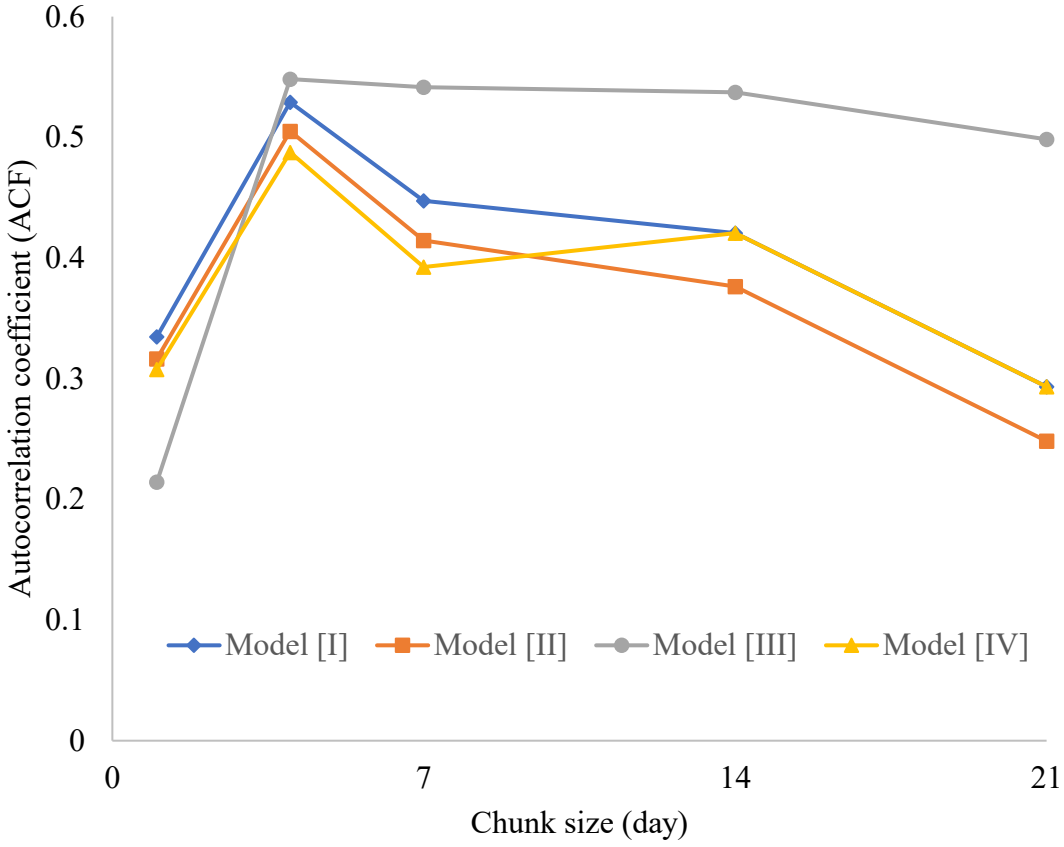

Figure 5. 1. Autocorrelation coefficient (ACF) of ME partitioning models in different chunk sizes of data (daily, 4-d, weekly, 2-wk, and 3-wk periods). Model [I]: $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$; where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); ε = the model residual. Model [II to IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for maintenance requirement, age, and ADG, respectively.

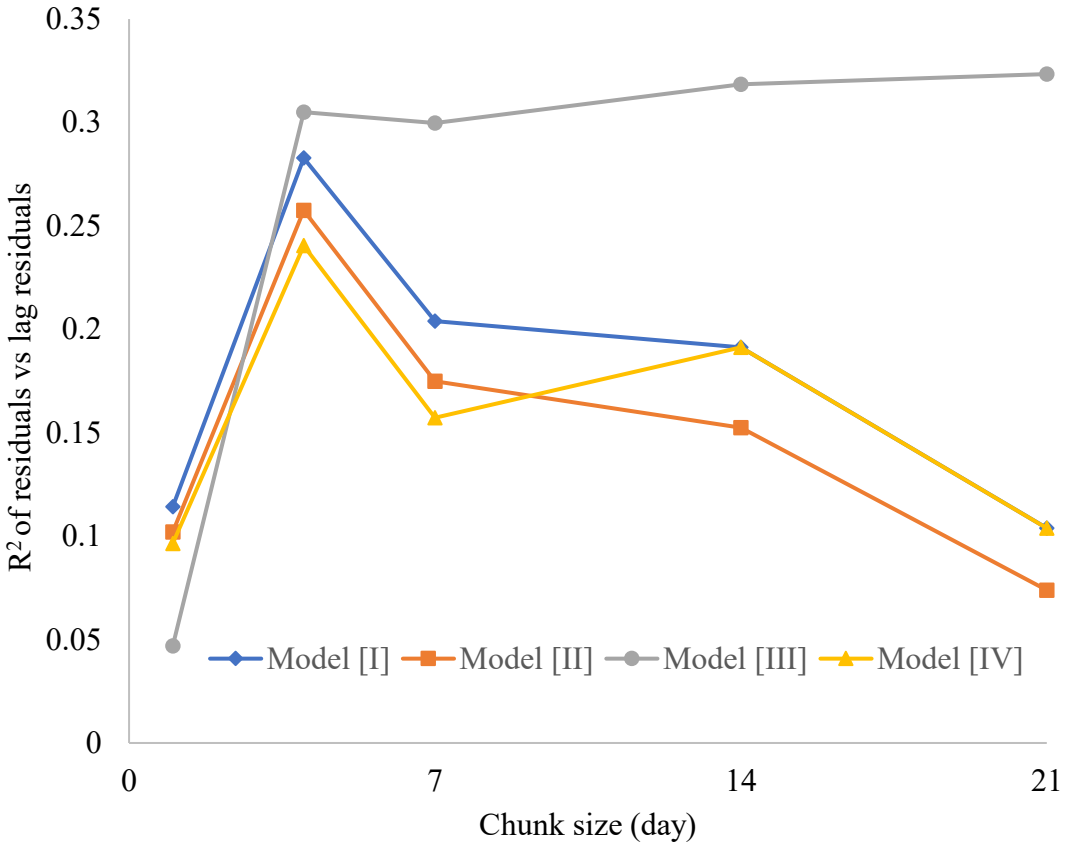

Figure 5. 2. R^2 of residuals vs lag residuals in ME partitioning models in different chunk sizes of data (daily, 4-d, weekly, 2-wk, and 3-wk periods). Model [I]: $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$; where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); ε = the model residual. Model [II to IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for maintenance requirement, age, and ADG, respectively.

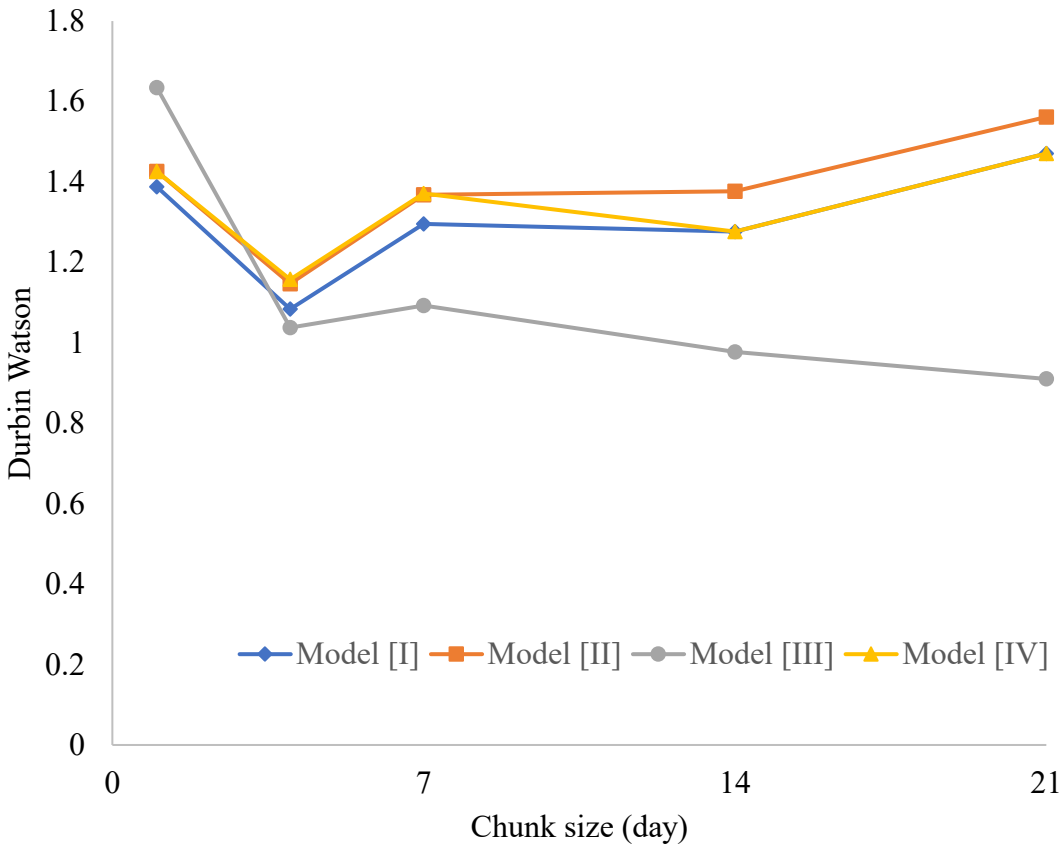

Figure 5. 3. Durbin Watson statistic of ME partitioning models in different chunk sizes of data (daily, 4-d, weekly, 2-wk, and 3-wk periods). Model [I]: $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$; where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); ε = the model residual. Model [II to IV] were nonlinear mixed models based on the function of model [I] with inclusion of a random term for maintenance requirement, age, and ADG, respectively.

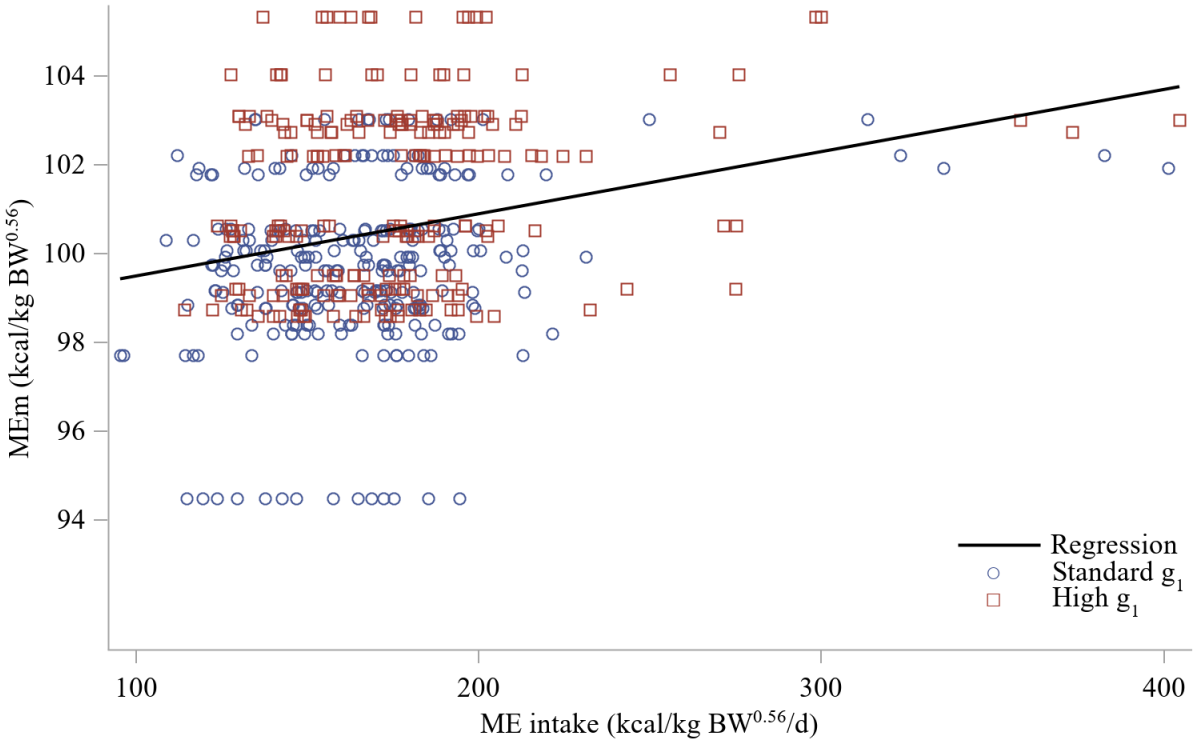

Figure 5. 4. Estimates of the individual maintenance requirement (ME_m) relative to average daily ME intake for the duration of the experiment (from 2 to 43 wk of age) as estimated by a mixed-effect model describing ME partitioning to maintenance, gain, and egg production in a 3-wk chunked data. The model was $MEI_d = (a + u) \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); u = bird-specific random term associated with individual maintenance. g_1 was either the gain coefficient for the prepubertal phase, estimated from the breeder-recommended standard BW gain (Standard g_1) target, or 10% higher (High g_1). Regression equation was $ME_m = 98.09 + 0.013 \times MEI + \varepsilon$ ($P < 0.001$, $R^2 = 0.073$).

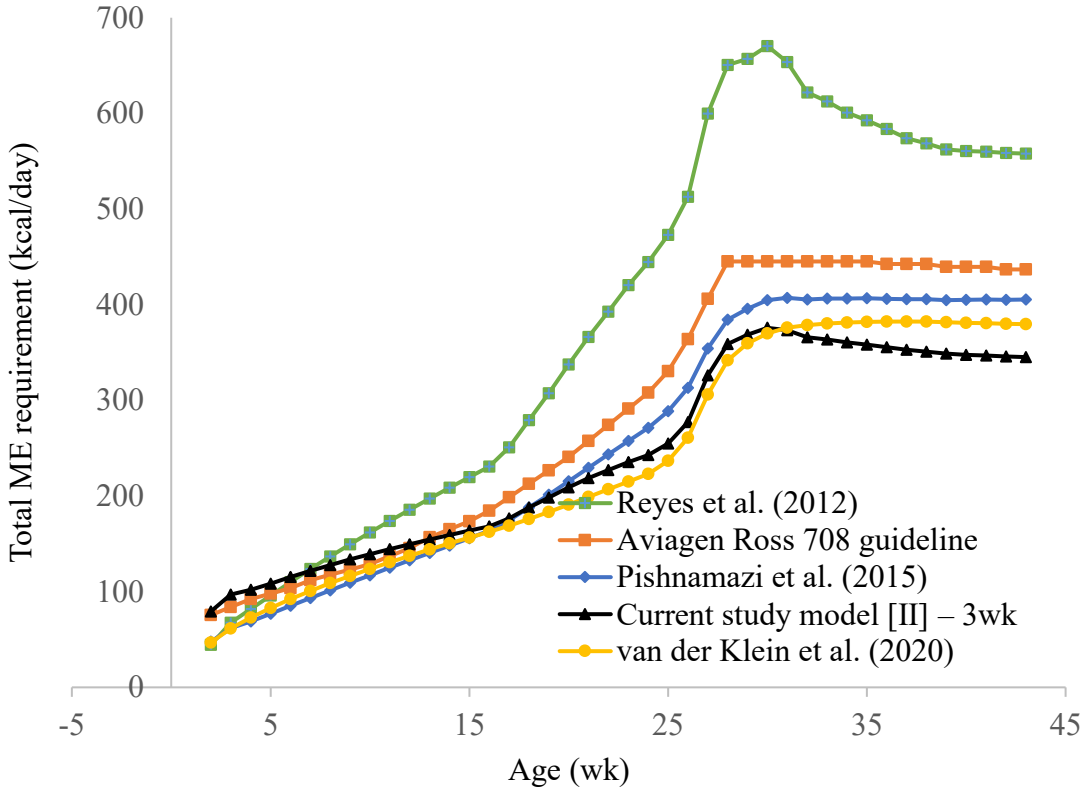

Figure 5. 5. Simulation of broiler breeder ME requirements by applying the Aviagen guide BW, ADG and egg mass (EM) data in the Reyes et al. (2012; ▪), Pishnamazi et al. (2015; ♦), van der Klein et al. (2020; ●), and the current study (model [II] – 3wk; ▲) models from 2 to 43 wk of age at 20°C environmental temperature. Ross 708 breeder guideline ME intake (■) was calculated by multiplying the guideline feed intake data by dietary energy (2,800 kcal/kg). Model [II] – 3wk was a mixed effect model with inclusion of a random term for individual maintenance ME in a 3-wk chunked data. The model was $MEI_d = (a + u) \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); u = bird-specific random term associated with individual maintenance.

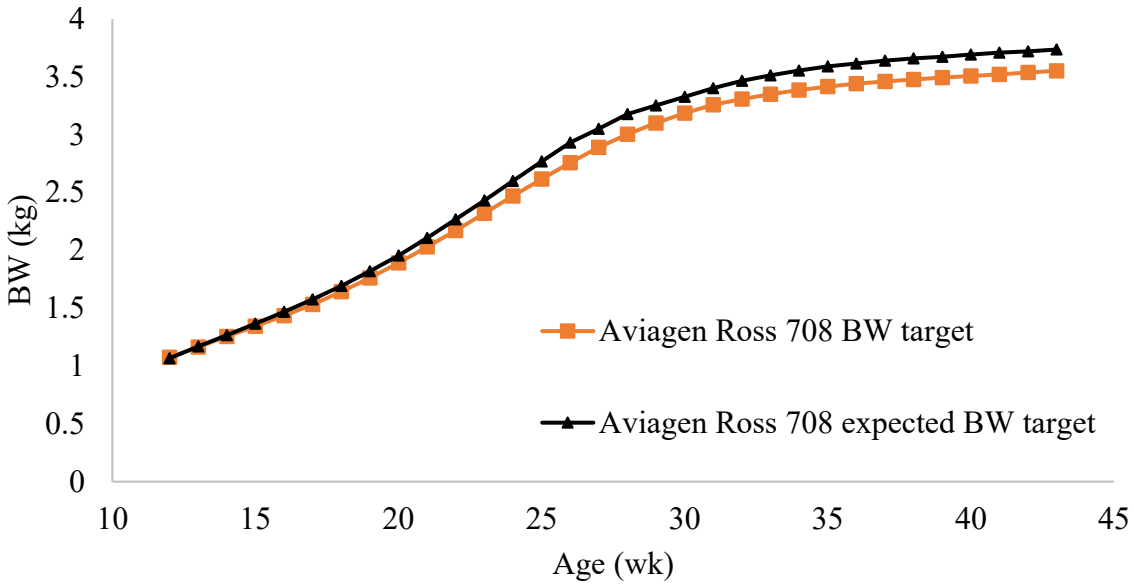

Figure 5. 6. Ross 708 broiler breeder recommended BW target (■) and expected BW target (▲) predicted by applying the guideline performance data in the current study ME partitioning model. The current study model was a mixed effect model describing ME partitioning to maintenance, gain, and egg production with inclusion of a random term for individual maintenance ME in a 3-wk chunked data. The model was $MEI_d = (a + u) \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + \varepsilon$, where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADG_n = negative ADG (g/d); EM = egg mass (g/d); u = bird-specific random term associated with individual maintenance.

6.0 Chapter 6: Intergenerational Effects of Maternal Growth Strategies in Broiler Breeders

6.1 Abstract

Maternal growth patterns affect broiler growth performance. The current study investigated the impact of lesser growth restriction, compared to the breeder-recommended target growth, during the pre-pubertal growth phase and earlier pubertal growth in breeders on their offspring growth and carcass traits. In a randomized controlled trial, a total of 40 female broiler breeders were randomly assigned to 10 unique growth trajectories with 2 levels of maternal BW gain (**MW**) in pre-pubertal phase and 5 levels of maternal pubertal growth inflection (**MI**) for each level of the MW. Growth parameters (MW and MI) were estimated by fitting a 3-phase Gompertz model to the breeder-recommended BW target (Standard MW; **SMW**), or 10% higher (**HMW**). Maternal pubertal inflection was advanced by 0, 5, 10, 15, or 20% in both SMW and HMW groups. Maternal growth trajectories were implemented from 0 to 42 wk of age using a precision feeding (**PF**) system. The current study consisted of two cohorts that varied in maternal age (**MA**) of 35 and 42 wk. The broiler chicks were fed to 35 d of age, also with the PF system. Analysis of covariance was conducted on all dependent variables (BW, FCR, carcass traits) with MA, MW, and offspring sex as categorical variables and MI as a continuous predictor variable. Chicks from 42 wk old hens had higher 0 (hatch), 14, 21, and 28 d BW, liver, and heart weights, and lower FCR from 7 to 35 d of age than those from the 35 wk old hens. Compared to SMW hens, HMW hens produced female offspring with lower FCR, and male offspring with heavier gut weight. Advancing MI increased hatch BW in both sexes and 35 d BW in male broilers. For every week that the MI was advanced, hatch BW increased by 0.26 g in females and 0.39 g in males; however, 21 and 35 d BW decreased by 6.85 and 17.29 g/wk in females and increased by

10.53 and 25.94 g/wk in males, respectively. Overall, a lesser degree of growth restriction during pre-pubertal and earlier pubertal growth increased male offspring growth.

Key words: broiler breeder, carcass, feed restriction, intergenerational, multi-phasic growth

6.2 Introduction

Controlling body weight in broiler breeders is achieved through feed restriction. The gap between growth potential of broilers and broiler breeder target BW has increased over the last 60 years (Renema et al., 2007). Thus, the intensity of broiler breeder feed restriction has increased which can impair reproductive performance (van der Klein et al., 2018; Zuidhof, 2018) and raise welfare concerns (van Krimpen and de Jong, 2014). The degree of feed restriction depends on the target growth curve; optimality of primary breeder-prescribed growth curves has rarely been reported. It is also valuable to investigate the intergenerational impact of lesser growth restriction and earlier pubertal growth.

Broiler growth rate, body composition, feed intake level, and skeletal health status are highly affected by their genetic potential (Havenstein et al., 2003). Breeder management practices, maternal age and maternal nutrition have also been reported to affect broiler performance (Triyuwanta et al., 1992; Kidd, 2003; Calini and Sirri, 2007; Enting et al., 2007). Most of the research pertaining to consequences of maternal effects in chickens have focused on nutrient composition of the diet; however, there is little data on effects of alterations of the maternal pre-pubertal BW gain (**MW**) and pubertal inflection (**MI**) on progeny performance in the literature. It has been reported that increasing target BW and the amount of feed available to broiler breeders increased offspring's hatch BW (van der Waaij et al., 2011) and final BW (van der Waaij et al., 2011; van Emous et al., 2015; Bowling et al., 2018).

Maternal feed restriction intensity can affect offspring abdominal fat deposition. van der Waaij et al. (2011) found that offspring of feed restricted breeders had significantly lower BW and relatively more abdominal fat deposition compared to those of breeders fed ad libitum. They concluded that it might be due to a mismatch between maternal and offspring feeding levels and nutritional environment which would potentially lead to economic loss and impaired feed efficiency. Humphreys (2020) observed heavier gut weight in broilers from 40-wk old hens that weighed 121% of the standard target BW compared to those of from standard BW hens.

The objective of the current study was to investigate the effect of a reduced degree of maternal pre-pubertal phase growth restriction and earlier maternal pubertal phase growth on offspring growth and development. It was hypothesized that increased MW and advanced MI would increase progeny hatch BW, final BW, and digestive tract weight, and lower MW would increase fat pad weight.

6.3 Materials and Methods

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

6.3.1 Maternal Study Design

In a randomized controlled trial, a total of 40 female Ross-708 broiler breeder pullets were randomly assigned to 10 growth trajectories (Figure 6.1) that were implemented using a precision feeding (**PF**) system. The maternal growth trajectories were designed using a 3-phase Gompertz model fit to the breeder-recommended target BW. The model had the form (Zuidhof, 2020):

$$BW_t = \sum_{i=1}^{i=3} g_i exp^{-exp^{-b_i(t-I_i)}} + \varepsilon_t$$

where BW_t was BW (kg) at time t (wk); g_i was the total amount of gain (kg) accruing in phase i ; b_i was the growth rate coefficient; t was age (wk); I_i was the inflection point (wk), or the age at which growth for phase i reached its maximum rate; and ε_t was the residual error with an expected value of 0, and a normally distributed variance estimated by the software $\varepsilon_t \sim N(0,SD^2)$; i was the growth phase (i = 1 to 3) where phase 1, 2, and 3 corresponded to pre-pubertal, pubertal, and post-pubertal growth phases. The maternal growth trajectories were designed with 2 levels of g_i in pre-pubertal phase as discrete variables; g_1 was either the estimated gain for phase 1 derived from the breeder-recommended standard BW (**SMW**) target, or 10% higher (**HMW**). The coefficient I_2, which biologically defined the inflection point of the pubertal growth phase, was advanced by 0, 5, 10, 15, or 20% of the coefficient estimated when fitting to the breeder-recommended target BW. I_2 was a continuous variable imposed in both the SMW and HMW groups. Each bird was an experimental unit.

6.3.2 Parent Stocks and Management

The pullets were housed in a single pen containing 2 PF stations, from hatch to 43 weeks of age at a stocking density of 3.0 birds per m^2. Water was provided ad libitum throughout the experiment. They were fed commercial diets: starter (crumble; AME 2,726 kcal/kg, 21.0% CP, 1.00% Ca, and 0.45% available P) from hatch to d 34; grower (mash; AME 2,799 kcal/kg, 15.0% CP, 0.79% Ca, and 0.44% available P) from d 35 to d 179; and laying diet (crumble; AME 2,798 kcal/kg, 15.3% CP, 3.30% Ca, and 0.38% available P) from 180 d onward. All birds were fed individually using a PF system (Zuidhof et al., 2019) that permitted feed intake levels appropriate to achieve the target growth trajectories of each individual bird. At 14 d of age each bird was equipped with a wing band containing a radio frequency identification (RFID)

transponder to be recognized individually by the PF system. The PF system provided access to a meal based on the individual pre-programmed target BW. If the BW exceeded the target BW, the system gently ejected the birds from the PF station. The birds had access to the PF system 24 hours per day throughout the experiment. Throughout the experiment, each time a bird entered the feeding station, its RFID, real-time BW and ADFI data (if fed) were recorded to a PF system database. Daily median BW for each individual bird were determined from database records of all visits to the PF station.

Settable eggs were collected from the experimental hens at 35 (cohort 1) and 42 wk of age (cohort 2) over 7 d prior incubation to conduct two separate offspring cohorts. These eggs were identified by hen and date, stored at 16°C and set into single-stage incubators with a randomized location. At 18 d of incubation, eggs were transferred to individual chick-hatching compartments with a newly randomized tray position.

6.3.3 Egg Components

Eggs were collected from every hen one week prior to cohort 1 (236 to 241 d) and cohort 2 (282 to 287 d) and immediately were used for egg proportion analysis. Eggs were separated into yolk, albumen, and shell. Dry weight of each component was determined after placing them in a drying oven at 60°C for 4 days.

6.3.4 Broiler Study Experimental Design

The progeny broiler study was designed as a completely randomized and controlled experiment. It included 2 replicated experiments that differed in maternal age (35 and 42 wk of age, which were called cohort 1 and 2, respectively). The experimental treatments were 10 unique maternal growth trajectories applied to the broiler breeders. Broilers were fed

individually using the PF system each time they entered the PF station. Therefore, each bird was an experimental unit.

6.3.5 Broiler Stocks and Management

Two offspring cohorts were conducted that differed in maternal age (**MA**): 35 and 42 wk of age. At hatch, chicks were feather-sexed, weighed, and identified with bar-coded neck tags (Heartland Animal Health Inc., Fair Play, MO). A total of 124 chicks (on average 12 chicks per maternal treatment) from each maternal age were randomly placed in environmentally controlled pens (n = 4) to 35 d of age. The initial set temperature was 32°C, which decreased by 1°C every 3 d until 22 °C. The photoperiod was 23L:1D (16 lx) from d 0 to 3 and decreased by 1 h of light each day until d 7 where the photoperiod remained at 19L:5D (8 lx) for the duration of the experiment. Wheat-corn-soybean-based diets were provided ad libitum in pelleted form as follows: starter (3,044 kcal of ME/kg; 23% CP; 1.27% Lys) from 0 to 11 d; grower (3,091 kcal of ME/kg; 22% CP; 1.18% Lys) from 11 to 21 d; and finisher (3,170 kcal of ME/kg; 21% CP; 1.13% Lys) from 21 to 40 d. Similar to the parent stock, the PF system recorded RFID, BW and FI data throughout the cohorts. All PF stations were turned off 12 h prior to euthanasia to achieve an empty gut weight. At 35 d of age, all broiler chicks were humanely euthanized and dissected. Breast muscle (pectoralis major and pectoralis minor), abdominal fat pad (including fat removed from the gizzard), liver, heart, and gastrointestinal tract (gut; 1 cm above the crop to the end of colon, adhering fat removed from the gizzard) weights were recorded.

6.3.6 Statistical Analysis

Analysis of covariance was conducted on all dependent variables using the MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC), with broiler sex, maternal age and

MW as sources of variation, MI as a continuous predictor variable, and dam as a random subject. In addition, one- and 2-way ANOVA were conducted using the MIXED procedure of SAS respectively on egg weight (**EW**) and maternal median BW (**MMBW**) during the period of egg collection for each progeny cohort at 35 and 42 wk of age to determine the relationship between EW and MMBW, and MI and MMBW. Pairwise differences between means were determined using Tukey's HSD test with the PDIFF option of the LSMEANS statement and were reported as different when $P \leq 0.05$. Trends were reported where $0.05 < P \leq 0.10$.

6.4 Results and Discussion

6.4.1 Egg Components

Eggs from 41 wk of age hens were heavier than those from 34 wk of age (P=0.006, Table 6.1). There was no effect of MW or MI on egg weight, dry eggshell, yolk, and albumen weight at either maternal age (Table 6.1). In the current study, trajectory-specific BW targets converged at 46 wk of age (Figure 6.1). Thus, target BW between BW trajectories differed more at 34 wk (a week prior to the egg collection period for the first cohort) compared to 41 wk (a week prior to the egg collection period for the second cohort). There was a negative relationship between MMBW and MI at 34 wk of age (-24 g/wk of advanced MI) which reduced by 41 wk (-14 g/wk of advanced MI), because the target growth trajectories were converged by 46 wk of age (Figure 6.1). Therefore, increasing MW or advancing MI did not increase EW at those ages. In consistence with the current study previous research showed that increasing target BW by 8% at 20 wk of age (Fattori et al., 1991), 20% at 18 wk of age (Hocking et al., 2002) 8% at 20 wk of age (van Emous et al., 2013), 16 and 20% at 20 wk of age (Gous and Cherry, 2004; Ekmay et al., 2012) did not affect average egg weight. However, in other research implementing higher target

BW by 21% (Renema et al., 2001a,b) and 13% (Sun and Coon, 2005) at 20 wk of age increased egg weight. In the current study however, MMBW was under the target BW in a hen causing a variation in MMBW inside the HMW treatment group. Thus, the effect of individual MMBW (regardless of the treatment) on EW of the eggs collected for each cohort was investigated. The results showed that increasing MMBW increased EW (P=0.001), and MA tended to increase the EW (P=0.064). It has been reported that egg size is an important factor in the chick weight, chick quality, and performance of broiler chicks to market weight (Abiola et al., 2008; Iqbal et al., 2016 and 2017) while others have found that any advantage of chicks hatched from large-sized eggs diminishes rapidly after hatching (Pinchasov, 1991; Yannakopoulos and Tserveni-Gousi, 1987).

6.4.2 BW and FCR

The chicks from 42 wk old breeders had higher 0 (hatch), 14, 21, and 28 d BW compared to those from 35 wk old hens (Table 6.2). Earlier MI increased hatch BW. For every week that the MI was advanced, hatch BW increased by 0.26 and 0.39 g in females and males, respectively. The effect of MI on BW at 21 and 35 d of age depended on sex (Figure 6.2). Specifically, males and females responded differently to MI. For every week that MI was advanced males 21, 28 and 35 d BW was increased by 10.53, 12.39 and 25.94 g, respectively. However, there was a 6.85, 12.45 and 17.29 g reduction in BW for females at those ages (Figure 6.2). When breeder-recommended target BW was increased by 121%, the final BW of offspring of HMW hens were 4% higher than those of SMW ones (Humphreys, 2020). In the current study, MW did not affect 35 d BW. Male broilers from breeders whose MI was advanced from 22 to 18 wk of age had greater BW on 21 and 35 d indicating a sex-dependent effect of MI on offspring BW. This may be related to sex-specific genetic potential, which affects body

composition (Zuidhof, 2005), plasma hormone levels (Gonzales et al., 2003), and muscle development (Henry and Burke, 1998). Bowling et al. (2018) found that increasing dam BW by 15% increased male broiler BW by 8.5% compared to that of the standard group. The authors further found that the concentration of yolk corticosterone of low BW hens was 1.2 times greater than that of high BW hens and suggested that males may be more sensitive to maternal feed restriction-induced stress. It is possible that in the current study, sex-dependent differences in 35 d BW might have been due to the reduced stress as a result of earlier MI and concomitant relaxed levels of feed restriction during the maternal pubertal phase.

For every week that MI was advanced, ADFI decreased by 0.92 and 0.03 g/d in female and male broilers, respectively. Average daily feed intake of HMW and SMW offspring respectively decreased by 18.33 and 19.33 g/wk of advanced MI (P = 0.040, Table 6.3). It has been shown that offspring of feed restricted might have higher ADFI (van Emous et al., 2015 in broiler breeders; Vickers et al., 2000 in rats). It is possible that in the current study, low maternal ADFI in SMW dams may have triggered induced reprogramming of genes that are responsible for feed intake through an epigenetic effect at a lower level of their offspring (van der Waaij et al., 2011).

Feed conversion ratio decreased in the second week compared to the first week studied (Table 6.3). Digestive tract maturation and development from 7 to 10 d may have resulted in poor utilization of nutrients, thus increasing FCR (Batal and Parsons, 2002). In addition, birds were being trained to use the PF system individually from 7 to 14 d of age, which may have decreased their ability to conserve energy and their feed intake. FCR of chicks from older breeders (42 wk) was lower than that of the ones of younger mothers (35 wk). Female broilers from HMW hens had a lower FCR from 7 to 35 d than that of SMW broilers (1.437 vs 1.444 g:g

for HMW and SMW broilers, respectively). This might have happened since female offspring from HMW hens had 0.69% lower ADFI over the course of 7 to 35 d and 5.8% lower abdominal fat deposition (Table 6.4) compared to their counterparts from the SMW hens; fat deposition in the body is energetically expensive, at approximately 9.2 kcal/g of BW gain, in contrast with lean tissue, which is composed of protein (4.1 kcal/g), and water (0 kcal/g; Zuidhof et al., 2014). For every wk that MI was advanced, FCR of female broilers of HMW decreased by 0.0001 g:g but increased 0.021 g:g for males (P=0.059). Notably, male chicks from HMW had greater gut weight compared to their SMW counterparts (P=0.048, Table 6.4) which could potentially increase the overall FCR by increasing maintenance cost of the digestive tract.

6.4.3 Carcass Components

Broilers from HMW hens tended to have heavier breast muscles than that of from the SMW hens (P=0.071, Table 6.4). A similar trend was observed on interaction of MI and sex on breast muscle weight (P=0.067). For every week that the MI was advanced, breast muscle weight increased by 4.9 g for males, and decreased by 6.12 g for females. This is consistent with findings of van Emous et al. (2015) and Spratt and Leeson (1987) that male and female offspring responded differently to maternal nutrition, which may be related to epigenetic sex-specific genes that affect body composition in the offspring (van der Waaij et al., 2011). However, no effect was seen on proportional breast as a percent of live BW (Table 6.5).

The current data showed that increasing MW by 10% or advancing MI did not reduce abdominal fat content. This did not support our hypothesis that the offspring fat deposition would increase as a result of lower nutrition level (SMW) in breeders. van der Waaij et al. (2011) and Jing-feng et al. (2014) demonstrated that offspring of feed restricted breeders had relatively more abdominal fat deposition compared to those of breeders fed ad libitum due to a mismatch

between maternal and offspring feeding level. It could be concluded that the maternal and offspring feeding level were not sufficiently different to reduce offspring abdominal fat pad weight in the current study. Since the goal of broiler production is to produce lean meat, an increase in broiler fat pad weight is not desired. Although SMW did not increase fat deposition in offspring broilers, based on the results of the current study, HMW still is recommended in broiler breeder industry due to its effect on reducing FCR in females.

Male broilers of HMW had a greater gastrointestinal tract (**GIT**) weight than those of SMW group, however, no effect was observed in females (Table 6.4). The gut is responsible for nutrient absorption, which plays a key role in metabolism to support growth and muscle development. A larger gut may have allowed the HMW hen offspring to make more efficient use of their feed due to the larger surface area of the gut, subsequently increasing their 35 d BW. Similarly, male broilers proportional GIT weight tended to increase by 0.08% of the live BW/wk of advanced MI (P=0.058, Table 6.5).

Chicks from 42 wk old breeders had higher liver and heart weights than those of 35 wk old hens (Table 6.4). Proportional liver weight of broilers from 42 wk of age breeders was on average 1.05 times greater than that of broilers from 35 wk of age (Table 6.5). Advancing MI tended to increase male broilers of HMW liver weight compared to that of SMW (P=0.057). For females and males, heart weight increased by 0.06 and 0.24 g/wk of advanced MI, respectively. The liver is an important metabolic organ that supports growth and development. A heavy BW broiler might have a larger liver to support greater maintenance requirements compared to a low BW broiler. Thus, greater liver weight might be related to greater BW on d 35 of male broilers from breeders whose MI was advanced.

The mechanism behind the effect of maternal environmental and nutritional conditions could either be through altered egg composition (O'Sullivan et al., 1991; Ekmay et al., 2013, 2014) or epigenetic mechanisms (Ferguson-Smith, 2011). Epigenetic effects can be passed onto the offspring. Epigenetic mechanisms are defined as alterations in the gene expression profile of a cell that are not caused by changes in DNA sequence; DNA methylation is an example of an epigenetic mechanism (Otterdijk and Michels, 2016; Pang et al., 2017). There was no effect of MW or MI on egg weight and egg components, differences in BW at hatch, 21, and 35 d of age suggest an epigenetic mechanism. Further research is needed to clarify the underlying mechanisms of maternal effects of less feed restriction on offspring growth performance and carcass composition, with specific emphasis on epigenetics. The results of this study have a substantial implication for broiler enterprise in terms of productivity of the progeny chicks.

To investigate the effects of maternal growth patterns downstream in the broiler supply chain, the current experiment focused on relaxed maternal growth restriction during the pre-pubertal growth phase and earlier pubertal growth in breeders on their offspring growth and carcass traits. To our knowledge, this is the first investigation of the maternal effects of strategically designed growth trajectories based on advancing the timing of the pubertal growth phase in breeders. Overall, the current results indicate that increasing maternal pre-pubertal phase BW gain by 10% and advancing maternal pubertal phase inflection from 22 to 18 wk of age can increase male broiler growth rate and some carcass components weight in offspring chicks.

6.5 Acknowledgment

Funding from Alberta Agriculture and Forestry (Edmonton, AB, Canada), Poultry Innovation Partnership (Edmonton, AB, Canada), scholarship support of Alberta Innovates and Alberta Advanced Education, technical assistance from Chris Ouellette and in-kind support for the precision feeding system from Xanantec Technologies, Inc. (Edmonton, AB, Canada) is gratefully acknowledged.

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6.7 Tables

Table 6. 1. Effects of maternal pre-pubertal BW (MW) and maternal pubertal growth inflection (MI) on egg weight, dry eggshell, yolk, and albumen weight from 40 to 41 wk of age

Effect	MA[1]	MW[2]	EW[3]	SEM	Eggshell	SEM	Egg yolk	SEM	Egg albumen	SEM
			g							
MW		S	61.78	0.56	5.51	0.04	9.51	0.07	4.53	0.03
		H	62.84	0.62	5.41	0.06	9.84	0.08	4.89	0.05
MA	35wk		61.11[b]	0.58	5.41	0.04	9.63	0.08	4.68	0.03
	42wk		63.51[a]	0.60	5.52	0.06	9.72	0.06	4.73	0.05
			g/wk							
MI			-0.43	0.35	0.003	0.022	-0.032	0.043	-0.029	0.021
MI × MW		S	-3.43	0.56	-0.56	0.89	0.44	1.24	-0.56	0.71
		H	-3.21	0.51	-0.53	0.044	0.43	0.062	-0.51	0.035
Source of variation			P-value							
MW			0.74		0.53		0.11		0.43	
MI			0.22		0.50		0.25		0.73	
MI x MW			0.67		0.61		0.72		0.20	
MA			0.006		0.12		0.42		0.37	

[a,b] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$); [1]MA: Maternal age; [2]MW: S=standard (breeder recommended) maternal pre-pubertal gain; H: maternal pre-pubertal BW gain 10% higher than the standard one; [3]EW: Egg weight

Table 6. 2. Effects of maternal pre-pubertal BW (MW), maternal pubertal growth inflection (MI), and offspring sex on BW at 0, 7, 14, 21, 28, and 35 d of broiler chickens

				BW (g)											
Effect	MA[1]	MW[2]	Sex[3]	0 d	SEM	7 d	SEM	14 d	SEM	21 d	SEM	28 d	SEM	35 d	SEM
MW		S		42.3	0.4	150	1.7	356	5.0	746	11.8	1,300	21.5	1,916	31.3
		H		43.6	0.4	152	1.9	359	5.3	755	11.6	1,323	22.4	1,942	31.0
Sex			F	43.0	0.4	150	1.8	352	5.0	734[b]	11.5	1,274[b]	21.1	1,857[b]	27.6
			M	42.9	0.4	152	1.8	363	5.3	767[a]	11.7	1,349[a]	22.5	2,001[a]	34.4
MW × Sex		S	F	42.6	0.5	150	2.4	354	6.9	738	16.5	1,274	28.1	1,853[b]	35.9
			M	42.0	0.6	149	2.5	358	7.1	753	16.3	1,325	32.2	1,979[ab]	52.2
		H	F	43.5	0.6	150	2.7	349	7.2	730	16.3	1,274	31.5	1,862[ab]	41.7
			M	43.8	0.4	154	2.7	368	7.8	780	16.6	1,372	31.7	2,022[a]	45.6
MA	35wk			42.3[b]	0.4	151	1.8	325[b]	4.7	684[b]	10.9	1,216[b]	20.8	1,903	30.8
	42wk			43.6[a]	0.4	151	1.9	390[a]	5.8	817[a]	13.0	1,407[a]	23.7	1,955	31.4
				g/wk											
MI x Sex			F	-0.26	0.322	-1.02	1.58	2.74	4.59	6.85	10.80	12.45	18.62	17.29	23.91
			M	-0.39	0.48	-0.82	2.20	-3.40	6.17	-10.53	14.35	-12.39	26.61	-25.94	39.66
MI x MW		S		5.17	9.74	0.12	0.47	75.61	12.99	84.07	29.85	256.45	54.63	408.62	71.25
		H		4.95	0.48	0.11	2.35	71.60	6.50	79.49	14.90	243.68	27.31	388.72	35.56
Source of variation				P-value											
MW				0.73		0.95		0.37		0.34		0.16		0.16	
MI				0.035		0.26		0.31		0.21		0.18		0.097	
MI x MW				0.89		1.00		0.38		0.37		0.18		0.18	
Sex				0.90		0.93		0.15		0.023		0.035		0.017	
MW x Sex				0.66		0.95		0.94		0.59		0.46		0.50	
MI x Sex				0.92		0.90		0.18		0.033		0.054		0.031	
MI x MW x Sex				0.61		1.00		0.99		0.65		0.50		0.52	
MA				0.017		0.84		< 0.001		< 0.001		< 0.001		0.24	

[a,b] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$); [1]MA: Maternal age; [2]MW: S=standard (breeder recommended) maternal pre-pubertal gain; H: maternal pre-pubertal BW gain 10% higher than the standard one; [3]Sex: F = Female; M = Male.

Table 6. 3. Effects of maternal pre-pubertal BW (MW), maternal pubertal growth inflection (MI), and offspring sex on FCR and daily feed intake at different ages of broiler chickens

Effect	MA[1]	MW[2]	Sex[3]	FCR (g/g)										ADFI (g/d)	
				7-14 d	SEM	14-21 d	SEM	21-28 d	SEM	28-35 d	SEM	7-35 d	SEM	7-35 d	SEM
				g/g										g/d	
MW		S		1.441	0.025	1.312	0.008	1.451	0.007	1.415	0.012	1.425	0.008	90.0	1.6
		H		1.448	0.027	1.322	0.009	1.450	0.007	1.425	0.010	1.430	0.007	91.1	1.5
Sex			F	1.467	0.027	1.329	0.008	1.459	0.007	1.427	0.011	1.441	0.007	87.9[b]	1.4
			M	1.422	0.025	1.305	0.008	1.442	0.007	1.414	0.011	1.414	0.008	93.2[a]	1.7
MW × Sex		S	F	1.460	0.032	1.326	0.010	1.461	0.010	1.435	0.016	1.444	0.010	88.2	1.9
			M	1.422	0.033	1.298	0.012	1.441	0.009	1.396	0.017	1.406	0.013	91.7	2.6
		H	F	1.474	0.037	1.332	0.013	1.457	0.010	1.418	0.015	1.437	0.010	87.6	1.9
			M	1.423	0.032	1.312	0.011	1.443	0.011	1.431	0.014	1.422	0.010	94.6	2.2
MA	35wk			1.641[a]	0.039	1.308	0.009	1.426[b]	0.008	1.448[a]	0.010	1.479[a]	0.009	92.6	1.5
	42wk			1.248[b]	0.016	1.326	0.008	1.475[a]	0.007	1.392[b]	0.012	1.376[b]	0.007	88.5	1.5
				g/g/wk										– g/d/wk –	
MI x Sex			F	-0.041	0.019	0.013	0.006	-0.0007	0.0068	-0.0051	0.0099	-0.0001	0.0062	0.92	1.2
			M	-0.029	0.026	0.005	0.009	-0.0025	0.008	0.016	0.013	0.021	0.009	0.03	2.04
MI x MW		S		-0.480	0.598	0.294	0.209	-0.073	0.191	0.0279	0.276	0.031	0.175	19.33	3.50
		H		-0.456	0.029	0.279	0.010	-0.069	0.009	0.0256	0.013	0.029	0.008	18.33	1.74

Source of variation	P-value					
MW	0.10	0.12	0.84	0.16	0.047	0.037
MI	0.051	0.22	0.47	0.56	0.21	0.17
MI x MW	0.098	0.14	0.83	0.18	0.051	0.040
Sex	0.25	0.65	0.37	0.051	0.12	0.032
MW x Sex	0.68	0.63	0.44	0.21	0.049	0.16
MI x Sex	0.29	0.54	0.30	0.060	0.18	0.049
MI x MW x Sex	0.70	0.61	0.46	0.27	0.059	0.18
MA	< 0.001	0.13	< 0.001	0.002	< 0.001	0.068

[a,b] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$); [1]MA: Maternal age; [2]MW: S=standard (breeder recommended) maternal pre-pubertal gain; H: maternal pre-pubertal BW gain 10% higher than the standard one; [3]Sex: F = Female; M = Male

Table 6. 4. Effects of maternal pre-pubertal BW (MW), maternal pubertal growth inflection (MI), and offspring sex on individual breast, fat pad, liver, heart, and gastro-intestinal tract (GIT) weight.

Effect	MA[1]	MW[2]	Sex[3]	Breast	SEM	Fat pad	SEM	Liver	SEM	Heart	SEM	GIT	SEM
				(g)									
MW		S		407	9.0	23.9	0.8	35.0	0.6	11.0	0.3	119.9	1.6
		H		416	8.2	23.8	0.9	35.0	0.7	11.2	0.3	120.5	1.6
Sex			F	407	8.6	25.2	0.8	33.1	0.6	10.1	0.3	114.1[b]	1.4
			M	416	8.3	22.6	0.9	36.8	0.7	12.1	0.3	126.3[a]	1.8
MW × Sex		S	F	409	12.0	25.9	1.0	33.3	0.7	9.9	0.3	114.1[b]	1.7
			M	404	13.7	22.0	1.3	36.6	1.1	12.2	0.5	125.6[a]	2.7
		H	F	404	12.2	24.4	1.3	32.9	0.9	10.3	0.4	114.0[b]	2.2
			M	428	10.4	23.2	1.3	37.1	1.0	12.0	0.3	126.9[a]	2.3
MA	35wk			405	9.1	23.1	0.9	33.8[b]	0.6	10.6[b]	0.3	119.0	1.5
	42wk			417	8.3	24.7	0.8	36.2[a]	0.7	11.6[a]	0.3	121.4	1.7
				g/wk									
MI x Sex			F	6.12	7.94	-0.11	0.68	0.30	0.46	-0.06	0.22	-0.60	1.12
			M	-4.9	11.46	0.74	1.03	-0.04	0.76	-0.24	0.35	2.62	1.99
MI x MW		S		201.39	22.10	0.29	2.14	10.21	1.45	5.89	0.67	-39.12	3.54
		H		191.11	11.02	0.20	1.07	9.69	0.72	5.62	0.33	-37.18	1.77

Source of variation	P-value				
MW	0.071	0.20	0.057	0.45	0.55
MI	0.11	0.62	0.15	0.047	0.39
MI x MW	0.080	0.19	0.057	0.46	0.56
Sex	0.058	0.95	0.058	0.45	0.93
MW x Sex	0.62	0.21	0.31	0.65	0.048
MI x Sex	0.067	0.92	0.11	0.72	0.73
MI x MW x Sex	0.69	0.24	0.33	0.70	0.051
MA	0.35	0.20	0.008	0.018	0.28

[a,b] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$) [1]MA: Maternal age; [2]MW: S=standard (breeder recommended) maternal pre-pubertal gain; H: maternal pre-pubertal BW gain 10% higher than the standard one [3]Sex: F = Female; M = Male.

Table 6. 5. Effects of maternal pre-pubertal BW (MW), maternal pubertal growth inflection (MI), and offspring sex on individual breast, fat pad, liver, heart, and gastro-intestinal tract (GIT) as a percent of live BW

Effect	MA[1]	MW[2]	Sex[3]	Breast	SEM	Fat pad	SEM	Liver	SEM	Heart	SEM	GIT	SEM
				(% of live BW)									
MW		S		21.09	0.21	1.23	0.03	1.82	0.02	0.58	0.01	6.39	0.12
		H		21.43	0.22	1.21	0.04	1.81	0.02	0.58	0.01	6.28	0.11
Sex			F	21.84	0.21	1.34	0.04	1.79	0.02	0.55	0.01	6.22	0.10
			M	20.69	0.22	1.11	0.04	1.84	0.02	0.61	0.01	6.45	0.13
MW × Sex		S	F	21.94	0.29	1.38	0.05	1.80	0.03	0.54	0.02	6.21	0.14
			M	20.24	0.31	1.09	0.05	1.85	0.04	0.61	0.02	6.56	0.21
		H	F	21.73	0.31	1.30	0.06	1.78	0.03	0.56	0.02	6.22	0.15
			M	21.13	0.32	1.12	0.06	1.83	0.03	0.60	0.02	6.33	0.17
MA	35wk			21.16	0.22	1.19	0.04	1.77[b]	0.02	0.56	0.01	6.34	0.10
	42wk			21.36	0.22	1.25	0.04	1.86[a]	0.02	0.60	0.01	6.32	0.14
				% of live BW/ wk									
MI x Sex			F	0.15	0.19	-0.007	0.031	0.004	0.017	-0.008	0.011	-0.08	0.09
			M	-0.01	0.26	0.044	0.042	0.017	0.027	-0.008	0.017	0.17	0.15
MI x MW		S		4.58	0.55	-0.155	0.096	0.120	0.052	0.218	0.033	-3.16	0.26
		H		4.35	0.27	-0.146	0.048	0.114	0.026	0.208	0.016	-3.01	0.13

Source of variation	P-value				
MW	0.19	0.33	0.48	0.65	0.28
MI	0.63	0.94	0.70	0.34	0.071
MI x MW	0.22	0.31	0.45	0.63	0.30
Sex	0.58	0.44	0.87	0.33	0.074
MW x Sex	0.89	0.23	0.69	0.18	0.69
MI x Sex	0.40	0.67	0.77	0.23	0.058
MI x MW x Sex	1.00	0.26	0.68	0.20	0.65
MA	0.51	0.25	0.009	0.069	0.89

[a,b] LSMeans within column and effect lacking a common superscript differ ($P \leq 0.05$) [1]MA: Maternal age; [2]MW: S=standard (breeder recommended) maternal pre-pubertal gain; H: maternal pre-pubertal BW gain 10% higher than the standard one [3]Sex: F = Female; M = Male

6.8 Figures

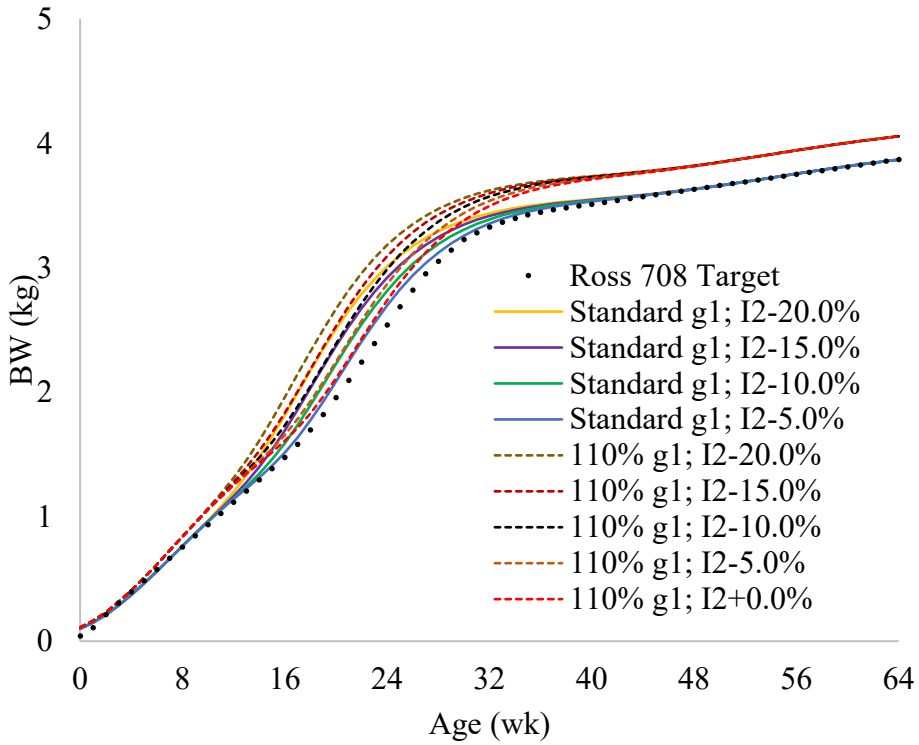

Figure 6. 1. Fit of a 3-phasic Gompertz model to target BW of Ross 708 broiler breeders (…) and increased total amount of gain (kg) in pre-pubertal growth phase (g_1) by 10% (---) with earlier pubertal growth inflection time (I_2) at 0, 5, 10, 15, and 20% of the standard BW curve.

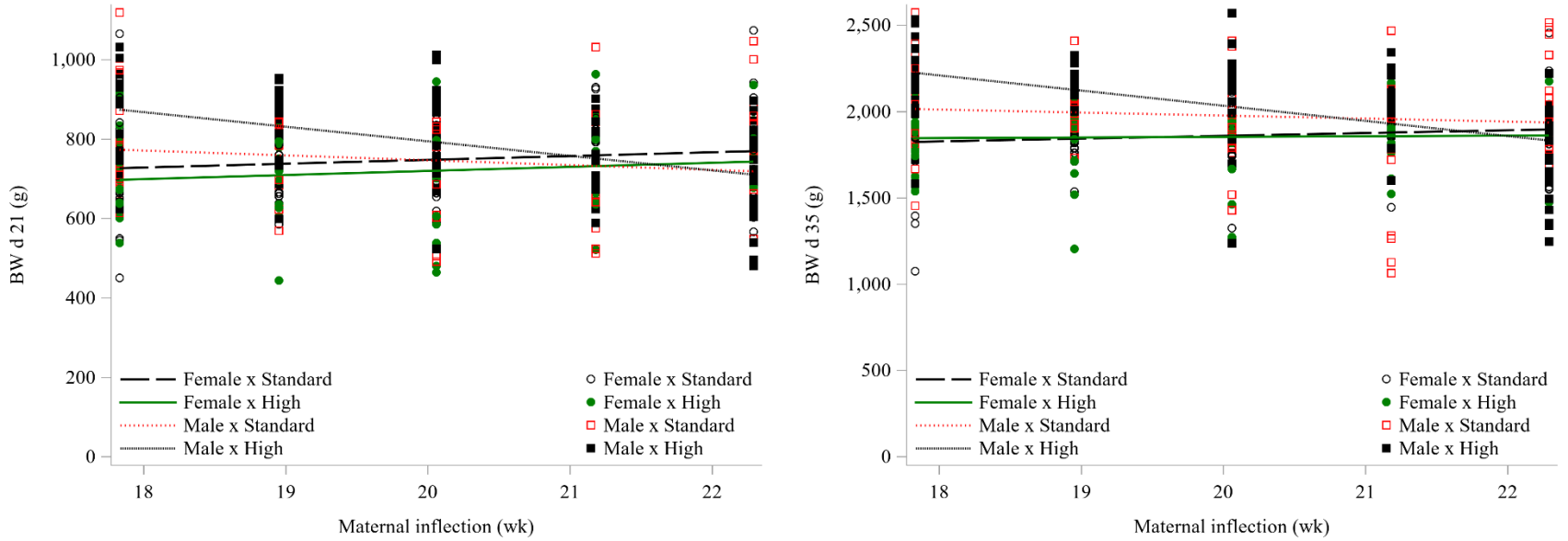

Figure 6. 2. Effects of maternal pre-pubertal growth (MW), maternal pubertal growth inflection (MI), and offspring sex on 21 and 35 d BW of offspring broilers.

7.0 Chapter 7: Plasma Metabolomic Profiling Reveals Potential Onset of Lay Biomarkers in Broiler Breeders

7.1 Abstract

Changes in the metabolic fingerprint of plasma during the onset of lay in broiler breeders were investigated. We used metabolomics to identify biomarkers of sexual maturity and to provide a comprehensive understanding of the metabolome of breeders during the pullet to hen transition period. A total of 36 pullets were used, in which 30 pullets were randomly assigned to one of 10 unique growth trajectories and 6 birds were assigned to an unrestricted group. The growth trajectories were designed using a 3-phase Gompertz growth model with 10 levels of BW gain in the prepubertal and pubertal growth phases ranging from the breeder-recommended target BW to 22.5% higher, in 2.5% increments. The BW trajectories were applied to each individual bird using a precision feeding (**PF**) system, which collected BW and feed intake data for each individual bird. The birds were classified based on age at first egg (**AFE**), and 12 pullets were chosen from the lower and upper AFE extremes (early and late onset of lay) at 18, 20, 22, 24, and 26 wk of age to run repeated blood plasma metabolomic assays. The metabolomic profile data were collected using a direct-injection liquid chromatography-tandem mass spectrometry and steroid assays. Univariate analysis identified 87 differential metabolites between the early- and late-onset of lay groups at 24 wk of age and 104 differential metabolites between the pullet and hen groups. Further investigation of differential metabolites showed 15 potential biomarkers for pullet to hen transition by analyzing the receiver operating characteristic (**ROC**) curve, mainly consisting of carnitine and choline metabolites. Differential metabolites during the pullet to hen transition were mainly associated with lipid, energy, and amino acid metabolism pathways. At 24 wk of age, the main pathways involved in differentiation of the early- and late-

onset of lay groups were related to lipid and amino acid metabolism. These metabolites could be involved in biosynthesis of egg yolk precursors in the liver.

Key words: broiler breeder, onset of lay, metabolomics, metabolic status, sexual maturation

7.2 Introduction

To successfully stimulate sexual maturity and persistent production upon photostimulation, broiler breeder pullets need to reach certain BW, body composition, and physiological thresholds within the context of metabolic balance (Sun and Coon, 2005; de Beer and Coon, 2009; van Emous et al., 2013; Sun et al., 2006). To date, most research models describing the control of the reproductive axis and sexual maturation have largely focused on the impact of environmental cues such as photoperiod (Bédécarrats, 2015), rather than incorporating the impact of growth trajectory and metabolic status. The impact of growth pattern on metabolism acts as a trigger for onset of lay in broiler breeders (Renema et al., 2007; Hanlon et al., 2020).

Broiler breeders are subjected to feed restriction programs (quantitative and qualitative) to control excessive growth during the rearing phase. Limited everyday feed restriction and skip-a-day feeding programs are the most common quantitative methods of feed restriction in broiler breeders (Carneiro et al., 2019). Diluting the nutrient density of the feed is an example of qualitative feed restriction (Zuidhof et al., 1995). The metabolic consequences of various restriction feeding regimes have been studied in broiler breeders (Buyse et al., 2000; Kita et al., 2002; de Beer et al., 2007, 2008; Ekmay et al., 2010; Moradi et al., 2013). Nutritional status and the subsequent responses of key plasma metabolic hormones (insulin, glucagon, and triiodothyronine) are important factors that determine the level of hepatic lipogenesis in birds (Hillgartner et al., 1995), which is involved in vitellogenesis. Although the length of the fasting

period is different in various feed restriction regimes, fasting is known to influence many metabolic processes, shifting metabolism from anabolism to catabolism and from lipogenesis to lipolysis (Richards et al., 2003). Likewise, feeding frequency can affect metabolic responses and reproductive efficiency. Variations in nutrient intake and subsequent energy status are communicated to the liver and hypothalamic-pituitary axis by alterations in the plasma levels of hormones such as insulin, glucagon, triiodothyronine and metabolites such as glucose, free fatty acids (Sun et al., 2006; de Beer et al., 2008). de Beer et al. (2007) found that skip-a-day feeding of broiler breeders was less efficient than everyday feeding due to the need to deposit and remobilize nutrients during the fasting period. Shortening fasting length, through increasing feeding frequency, increased feed utilization efficiency and enhanced egg production rate and egg weight, as well as reduced hepatic lipogenesis (Richards et al., 2003; Moradi et al., 2013). The liver provides amino acids, lipids, nucleotides, vitamins, and choline as essential compounds for yolk precursor synthesis (Zhu et al., 2020). The authors indicated that plasma glutathione and ascorbic acid levels were downregulated, and choline abundance was upregulated during onset of lay in ducks, which can be used to predict sexual maturity.

The effects of reproductive system maturation and the reproductive hormones produced turn a pullet into a hen. Sexual maturity is most commonly measured as age at first egg (**AFE**) in poultry (Renema et al., 2007; Wolc et al., 2010; Hadinia et al., 2020). However, development of medullary bone and ovarian follicles are initiated roughly 14 to 16 days before the first oviposition (Whitehead, 2004; Shi et al., 2020), which indicates another measure for sexual maturity. The process of sexual maturation in breeder hens embodies a major shift in their physiological status (Johnson et al., 2009). Fluctuations in plasma hormones and substrates may provide signals that link metabolic status to the activation of the reproductive system. The

maturation involves activation of the hypothalamus-pituitary-gonad (**HPG**) axis in which is controlled by hypothalamic secretion of Gonadotropin Releasing Hormone (**GnRH**). Hadinia et al. (2020) increased broiler breeder dietary energy from 2,807 to 3,109 kcal/kg of diet from 22 to 26 wk of age. The percentage of birds which commenced laying was 100% in the high ME intake treatment and 30% in the low ME intake treatment. They concluded that higher ME intake advanced the activation of HPG axis, stimulated reproductive hormone levels, and increased lipid deposition in the body of high ME intake treatment group.

Previously, we investigated the effects of incremental increases in target BW gain, including non-restricted broiler breeders, during prepubertal and pubertal growth phases on reproductive performance (Zukiwsky et al., 2021). The onset of lay depended on the degree of feed restriction, and some of the unrestricted pullets commenced egg production 2 wk prior to photostimulation. These results strongly suggest that body composition, or metabolic status, or both have a role in triggering sexual maturation. In the current study we have investigated the metabolomic alterations in the broiler breeder plasma around the onset of lay. At present, most metabolomic studies have focused on mammals, and little is known about the metabolomics of broiler breeders. Therefore, profiling the plasma metabolome may provide a new perspective for studying the metabolic response of sexual maturity in breeders, a better understanding of its biological mechanisms, and provide potential biomarkers for predicting the onset of lay. The objectives of the current study were to evaluate the effect of lay status (pullet vs. hen), photostimulation BW, and onset of lay timing (early vs. late) on plasma metabolomic dynamics to identify potential biomarkers of sexual maturity.

7.3 Materials and Methods

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

7.3.1 Animals and Management

The experimental protocol was previously described in full detail (Zukiwsky et al., 2021). Briefly, a total of 36 (30 growth restricted and 6 unrestricted) Ross 708 broiler breeder pullets were placed in a single pen from hatch to 43 wk of age at a stocking density of 3.0 birds per m^2. The birds were fed using 2 precision feeding (**PF**) stations, which collected real-time BW and feed intake data for each individual bird. All birds were fed commercial broiler breeder diets: starter (crumble; ME 2,726 kcal/kg, 21% CP, 1.00% Ca, and 0.45% available P) from hatch to d 34; grower (mash; ME 2,799 kcal/kg, 15% CP, 0.79% Ca, and 0.44% available P) from d 35 to d 179; and laying diet (crumble; ME 2,798 kcal/kg, 15.3% CP, 3.30% Ca, and 0.38% available P) from d 180 onward. Water was provided ad libitum throughout the experiment. The photoschedule was 8L:16D (15 lx) during the rearing phase. Pullets were photostimulated at wk 22 by increasing the photoperiod to 11L:13D (20 lx); to 12L:12D (25 lx) on wk 23, then at wk 24 to 13L:11D (50 lx) for the remainder of the experiment. A nest box with 8 nesting sites equipped with radio frequency identification (**RFID**) readers, which identified and weighed eggs of individual hens, was installed in the room. To prevent floor eggs, sufficient additional laying space was provided as trap nests with 8 nesting sites, which also allowed the identification of each egg according to the hen that laid it. The nest systems were installed at 14 wk of age; so that the pullets had the chance to adapt to the nesting system prior to the onset of lay.

The PF system was used to identify individual birds using a wing band containing a RFID transponder and feed them according to how their real-time BW compared to the pre-programmed target BW (Zuidhof et al., 2019). The PF system provided access to a meal if the individual birds' real-time BW was equal to or less than its pre-programmed target BW; otherwise, the system gently ejected the birds from the PF station. All birds had access to the PF stations for 24 hours per day throughout the experiment.

7.3.2 Photostimulation BW and Age at First Egg

Median BW of the multiple BW observations of individual birds at 154 d of age (22 wk) were considered as their photostimulation BW. These data were extracted from the PF system database. To determine AFE, the cloacae of all hens were palpated daily to detect the presence of a hard-shelled eggs in the shell gland from 20 wk onward. Presence or absence of a hard-shelled egg in the shell gland was recorded for each hen to determine AFE. Eggs were collected from nest boxes, weighed, and individual hen laying records were reconciled daily.

7.3.3 Plasma Samples Preparation

Blood samples (3 mL) were taken from the brachial vein of each bird biweekly from 18 to 26 wk of age. A 4 mL sodium heparin vacutainer was used to collect blood from each bird between 1 to 3 h after the start of the photoperiod. The samples were immediately centrifuged at 1,244 x g at 4°C for 15 min to recover plasma. The plasma samples were stored at –20°C until metabolomic profile analysis. The metabolomic data were collected using a locally developed direct-injection liquid chromatography-tandem mass spectrometry (**DI/LC-MS/MS**) assay and steroid assay (Zwierzchowskia et al., 2020).

7.3.4 Experimental Design

The experiment was a completely randomized controlled study with 30 pullets reared on one of 10 growth trajectories. A 3-phase Gompertz growth model describing the growth in prepubertal, pubertal, and post-pubertal phases was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the phase-specific BW gain coefficients. The growth trajectories were designed with 10 levels of BW gain in the prepubertal and pubertal growth phases ranging from the breeder-recommended target BW (**CON**) to 22.5% higher (CON+22.5%) in 2.5% increments. The BW trajectories were applied to each individual bird using the PF system. Therefore, each bird was an experimental unit. An additional 6 birds were assigned to an unrestricted group. The unrestricted birds were not limited to a maximum BW or a certain growth trajectory but were rather provided access to feed upon every PF station visit.

The various BW trajectories in this study caused birds to commence laying at different ages, creating a range for AFE criteria. We used AFE to create 4 experimental classifications. 1) Early vs. late onset of lay: After collecting plasma samples, the candidate plasma samples were chosen for the metabolomic assays based on the bird's AFE. More specifically, 12 birds each having the highest and lowest AFE were selected for the metabolomics study. 2) Heavy vs. standard BW: The median photostimulation BW of the candidate birds was used to define the upper (heavy BW) and lower (standard BW) extremes. 3) Pullet vs. hen: The candidate birds were categorized as either a pullet or a hen at each blood sampling age, depending on whether they had laid an egg prior to the collection of the plasma sample. 4) Mature vs. immature birds: We subtracted 15 days from AFE as an estimated time for initiation of maturity in the birds. Then the candidate birds were divided into either mature or immature at each blood sampling age. Initiation of sexual maturity including the development of medullary bone and ovarian

follicles start around 14 to 16 days before the first oviposition (Whitehead, 2004; Shi et al., 2020).

7.3.5 Direct-Injection Liquid Chromatography-Tandem Mass Spectrometry

A targeted quantitative metabolomics approach was applied to analyze the plasma samples using a combination of direct injection mass spectrometry with a reverse-phase LC-MS/MS custom assay. This custom assay, in combination with an AB Sciex 4000 QTrap® (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) mass spectrometer, can be used for the targeted identification and quantification of up to 150 different endogenous metabolites including amino acids, acylcarnitine, biogenic amines and derivatives, uremic toxins, glycerophospholipids, sphingolipids, and sugars (Foroutan et al., 2019; 2020). The method combined the derivatization and extraction of analytes, and the selective mass-spectrometric detection using multiple reaction monitoring pairs. Isotope-labeled internal standards and other internal standards were used for metabolite quantification. The custom assay contained a 96 deep-well plate with a filter plate attached with sealing tape, reagents, and solvents used to prepare the plate assay. The first 14 wells were used for 1 blank, 3 zero samples, 7 standards, and 3 quality control samples. For all metabolites except organic acids, plasma samples were thawed on ice and were vortexed and centrifuged at 13,000 x g. Ten µL of each sample was loaded onto the center of the filter on the upper 96-well plate and dried in a stream of nitrogen. Subsequently, phenyl-isothiocyanate was added for derivatization. After incubation, the filter spots were dried again using an evaporator. Extraction of the metabolites was then achieved by adding 300 µL of extraction solvent. The extracts were obtained by centrifugation into the lower 96-deep well plate, followed by a dilution step with mass spectrometry running solvent (0.2% formic acid in

water, 0.2% formic acid in acetonitrile for biogenic amines and amino acids, and 0.02% formic acid in methanol for all other classes of metabolites).

For organic acid analysis, 150 µL of ice-cold methanol and 10 µL of isotope-labeled internal standard mixture were added to 50 µL of plasma samples for overnight protein precipitation. The samples were then centrifuged at 13,000 x g for 20 min. After that, 50 µL of supernatant was pipetted into the center of wells of a 96-deep well plate, followed by the addition of 3-nitrophenylhydrazine reagent. After incubation for 2 h, BHT stabilizer and water were added before LC-MS injection.

Mass spectrometric analysis was performed on an AB Sciex 4000 QTrap® tandem mass spectrometry instrument equipped with an Agilent 1260 series UHPLC system (Agilent Technologies, Palo Alto, CA). The samples were delivered to the mass spectrometer by a LC method followed by a direct injection method. Data analysis was done using Analyst 1.6.2 (Sciex Canada, Concord, ON).

7.3.6 Steroid Assay

7.3.6.1 Sample Preparation. Plasma samples were thawed on ice, in the darkness, before use. Then 100 µL of the samples (PBS as blank sample, calibration standards, quality control standards and plasma samples) was mixed with 20 µL of internal standards mixture solution and were pipetted into Eppendorf tubes. After that, 100 µL of PBS buffer was added to each tube and vortexed for 30 s. Then 1,000 µL of methyl tert-butyl ether was added to each tube for extraction. The samples were shaken for 15 min. Subsequently, samples were centrifuged at 13,000 x g and 4°C for 15 min, and 750 µL of supernatants were transferred into HPLC vials and dried under nitrogen purge until completely dry. To the dried tubes, 100 µL of derivatization solution (1.5 M Hydroxylamine in HPLC grade water) was added, followed by shaking for 15

min. All the tubes were then incubated at 60°C for 1 h, and subsequently 20 µL was injected into an UHPLC-equipped 4000 QTrap® mass spectrometer for LC-MS/MS analysis.

7.3.6.2 LC-MS/MS Method. An Agilent 1260 series UHPLC system (Agilent, Palo Alto, CA) was used for LC-MS/MS analysis with an AB Sciex 4000 QTrap® mass spectrometer (Sciex Canada, Concord, ON). The controlling software for the LC-MS system was Analyst 1.5.2. For the HPLC work, solvent A was 0.1% formic acid in water; and solvent B was 0.1% formic acid in methanol. The gradient profile for the UHPLC solvent run was set as follows: t = 0 min, 10% B; t = 1.50 min, 10% B; t = 2.50 min, 55% B; t = 7.50 min, 95% B; t = 8.50 min, 95% B; t = 8.60 min, 10% B; and t = 12.0 min, 10% B. The flow rate was 0.5 mL/min and the sample injection volume was 20 µL. The mass spectrometer was set to a positive electrospray ionization mode with multiple reaction monitoring. The Ion Spray voltage was set at 5,500 V and the temperature at 550°C. The curtain gas, ion source gas 1, ion source gas 2, and collision gas were set at 40, 60, 60 and medium, respectively. The entrance potential was set at 10 V. Likewise, the decluttering potential, collision energy, collision cell exit potential, multiple reaction monitoring Q1 and Q3 were set individually for each analyte and internal standards.

7.3.7 Statistical Analysis

All statistical analyses were performed for each blood sampling age separately. However, the analyses for the pullet vs. hen groups and the mature vs. immature birds' groups data were done for all blood sampling ages together to investigate the overall metabolic status of the groups. The MetaboAnalyst software (The Metabolomics Innovation Centre, Canada, AB) was used for the statistical analyses (Xia et al., 2009). After uploading the metabolomic profile data to the software and conducting an integrity check, metabolites that were frequently (> 20%) below the limit of detection or with more than 20% missing values were excluded from datasets.

Otherwise, missing values were estimated using the KNN (feature-wise) option of the software. The data were then normalized either by sum or median to reach a bell-shaped Gaussian distribution curve prior to statistical analyses. Univariate analysis methods including the fold change (**FC**) analysis, t-test, and volcano plot were conducted for exploratory data analysis. The univariate analyses provided a preliminary overview about compounds that were potentially significant in discriminating the effects under study. Metabolites with a FC value greater than 1.5 were considered as differential metabolites between the groups. Pairwise differences between metabolites concentrations within each group were reported as significantly different when $P \leq 0.05$. Trends were reported where $0.05 < P \leq 0.10$. Principal Component Analysis (**PCA**), an unsupervised pattern recognition method, was used to provide an overview of the population structure and to ensure clustering of the pooled quality controls. Additionally, Partial Least Squares - Discriminant Analysis (**PLS-DA**) model was employed for further robust separation of differential metabolites between two groups. Furthermore, the variable importance in the projection (**VIP**) values were used to define significantly differential metabolites (VIP > 1), i.e., metabolites with significant difference in concentration, between the groups.

The goodness of fit explains how well we were able to mathematically reproduce the data of the data set. A quantitative measure of the goodness of fit was given by the parameter R^2 (the explained variation, goodness of fit). The cross-validation method employed for this study was the 10-fold cross validation, with Q^2 as measured predictive performance (goodness of prediction). The PLS-DA model needs to be validated to confirm whether the separation is statistically significant, or due to random noise (Barberini et al., 2016). Thus, a 100 times permutation test was implemented to assess the significance of class discrimination and to validate the reliability of the PLS-DA model. More specifically in each permutation, a PLS-DA

model was built between the data (X) and the permuted class labels (Y) using the optimal number of components determined by previous cross validation calculations and based on the original class assignment. The ratio of the between sum of squares and the within sum of squares (**B/W-ratio**) was calculated for the class assignment prediction of each model. If the B/W ratio of the original class assignment were a part of the distribution based on the permuted class assignment, the contrast between the two class assignments could not be considered significant.

The Orthogonal Partial Least-squares Discriminant Analysis (**OPLS-DA**) was performed to further investigate and analyze the separation of the groups (Trygg et al., 2007). To determine the optimal potential biomarker for each group (the pullet and hen group and the early and late onset of lay group) receiver operating characteristic (**ROC**) curve and area under the curve (**AUC**) analyses were performed based on the cross-validation strategy. The ROC curves were generated by Monte-Carlo cross-validation using balanced subsampling. In each cross-validation, two thirds of the samples were employed to evaluate the differential compounds importance. The top 50 important compounds were then exploited to build classification models, which were validated on the remaining one-third of the samples. The procedure was replicated multiple times to calculate performance, and confidence interval of each model. The linear support vector machine algorithm was used as a classification and a feature ranking method with 2 latent variables. The following decision criteria were used: the AUC of 0.9 to 1.0 indicated excellent performance; 0.8 to 0.89, good performance; 0.7 to 0.79, fair performance; 0.6 to 0.69, poor performance; and less than 0.6, insignificant value (Haase-Fielitz et al., 2009). In addition, commercial databases including the Kyoto Encyclopedia of Genes and Genomes (**KEGG**) and *Gallus gallus* (chicken) metabolome database (KEGG database, 2019) were employed to further search for metabolite pathways associated with the significantly differential metabolites. These

compounds were then imported into the module of pathway analysis in MetaboloAnalyst to generate the pathway topology analysis. The metabolic pathway with an impact value greater than 0.1 was characterized as the significantly relevant pathway (Liu et al., 2018).

7.4 Results

7.4.1 Validation of Metabolomic Profile Models

Validation plots (fitting and predictive performance plot and permutation test plot) were used to validate the metabolomic models at different ages (18, 20, 22, 24, and 26 wk) and different treatments (pullet vs. hen, early vs. late onset of lay, heavy BW vs. standard photostimulation BW, mature vs. immature birds). Among all treatments and ages, only the metabolomic models resulting from the early vs. late onset of lay groups at 24 wk of age and the pullet vs. hen groups showed reliable fitting and predictive performance ($Q^2 > 0.40$; Worley and Powers, 2013; Blasco et al., 2015). As the grouping based on the heavy and standard photostimulation BW was equivalent to the grouping based on the early and late onset of lay, the metabolomic results of those groups can be interpreted together. The Q^2 values for the models resulted from the early vs. late onset of lay groups at 18 and 20 wk of age were less than 0.40 (0.002 for 18 wk and 0.005 for 20 wk of age), indicating unreliable predictive performance. Furthermore, the univariate analysis results for metabolomic data at 22 and 26 wk of age did not show any significant differences in metabolite concentrations between the early and late onset of lay groups, indicating the same metabolic status between the groups at those ages. The average AFE in the early and late onset of lay groups were 22 and 26 wk of age, respectively. Thus, it was speculated that the lack of differential metabolites before and after 24 wk of age would indicate a relatively stable physiological state before and after sexual maturation in the breeders.

7.4.2 Photostimulation BW and Age at First Egg

In our previous publication, we indicated that sexual maturity advanced by 10.8 d per kg increase in photostimulation BW (Zukiwsky et al., 2021). The birds' AFE ranged from 141 to 186 d of age with a median value of 175 d of age. In the current study, 6 birds with an AFE of less than the median AFE (lower extreme of AFE) were considered as the early onset of lay group, and 6 birds with an AFE of greater than the median AFE (upper extreme of AFE) were considered as the late onset of lay group. The late onset of lay group included birds from the CON, CON+2.5%, CON+5%, CON+10%, and CON+12.5% treatments and the early onset of lay group included birds from the CON+15%, CON+17.5%, CON+20%, and unrestricted treatments. The candidate birds' photostimulation BW ranged from 2,350 g (CON group) to 4,940 g (unrestricted group) with a median value of 2,675 g. Thus, birds with a photostimulation BW greater and lower than the median value were considered as the heavy and standard BW groups, respectively.

7.4.3 Plasma Metabolomic Profile

Using the DI/LC-MS/MS and steroid assays, a total of 142 metabolites (134 by DI/LC-MS/MS and 8 steroids) were identified and quantified in the plasma samples. The species of metabolites measured by the DI/LC-MS/MS were classified into six groups: amino acids (23), biogenic amines (16), organic acids (18), lyso-phosphatidylcholines acyl (14), hydroxy-sphingomyelins (10), phosphatidylcholines di-acyl (8), phosphatidylcholines acyl-alkyl (2), acyl-carnitines (40), hexose (1), and miscellaneous (2). The steroids were progesterone, corticosterone, 17-hydroxy progesterone, estrone, pregnenolone, testosterone, androstenedione, and dehydroepiandrosterone.

7.4.3.1 Pullet and Hen Groups. Univariate analyses provided the FC analysis, t-test, and volcano plot, which was a combination of the first two methods. Fifteen important compounds were identified by the FC analysis and volcano plot (Table 7.1), and 104 important compounds were identified by the t-test (top 50 compounds shown in Table 7.2) between the pullet and hen groups. Volcano plot analysis, which combines the FC and t-test analyses, revealed that the abundance of all identified plasma metabolites was lower in the hen group compared to the pullet group except for the plasma acetyl carnitine, creatine, and phosphatidylcholine metabolites (PC38:6AA, PC36:0AA, and PC40:6AA), which were upregulated.

7.4.3.2 Early and Late Onset of Lay Groups at 24 wk of Age. Eighteen important compounds were identified by the FC analysis and volcano plot (Table 7.3), and 87 important compounds identified by the t-test (top 50 compounds shown in Table 7.4) between the early and late onset of lay groups at 24 wk of age. Based on the volcano plot analysis, all the identified plasma metabolites were downregulated in the early compared to the late onset of lay group except for the plasma corticosterone and phosphatidylcholine metabolites (PC38:6AA and PC36:6AA), which were upregulated.

7.4.4 Principal Component Analysis of Samples

7.4.4.1 Pullet and Hen Groups. The PCA score plot showed a fairly clear separation between the pullet and hen groups. First and second principal components (PC1 and PC2) explained 43.4 and 10.4% of the variation in samples, respectively (Figure 7.1 panel A).

7.4.4.2 Early and Late Onset of Lay Groups at 24 wk of Age. The PCA score plot showed a clear separation between the early and late onset of lay groups at 24 wk of age. First and second principal components (PC1 and PC2) explained 57.9 and 8.9% of the variation in samples, respectively (Figure 7.1 panel B).

7.4.5 Partial Least-Squares Discriminant Analysis and Orthogonal Partial Least-Squares Discriminant Analysis of Plasma Samples

7.4.5.1 Pullet and Hen Groups. A PLS-DA and OPLS-DA model was constructed to further investigate and analyze the separation of the pullet and hen groups. As shown in Figure 7.2, the pullet and hen groups were clearly separated. First and second principal components (PC1 and PC2) explained 42.3 and 10.7% of the variation in samples in the PLS-DA score plot and 23.5 and 25.9% of the variation in samples in the OPLS-DA score plot (Figure 7.3 panel A).

The performance scores of the PLS-DA model for the pullet and hen groups were accuracy = 0.90, R^2 = 0.80, and Q^2 = 0.66 (Figure 7.2 panel C) and the performance scores of the OPLS-DA model were R^2Y cumulative (cum) = 0.489 and Q^2 cumulative (cum) = 0.447 (Figure 7.3 panel B), which were indicative of robust fit and prediction. R^2Y is the fraction of the variance of descriptor matrix (X) and class response (Y) explained by each latent variable in % representing explained variation in Y by the component. To further validate the PLSA-DA model, the permutation test (n = 100 times) was used for verification (Figure 7.2 panel D); the highlighted bar represents the original sample. The further to the right of the distribution, the more significant is the separation between the two groups (Bijlsma et al., 2006). The permutation test results of the OPLS-DA model for the R^2Y(cum) and Q^2(cum) values were 0.801 and 0.696 between the pullet and hen groups (Figure 7.3 panel C). Permutation test revealed that the observed separation was not by chance, and the results of cross-validation were reliable.

7.4.5.2 Early and Late Onset of Lay Groups at 24 wk of Age. The PLS-DA and OPLS-DA models showed a clear separation between the early and late onset of lay groups at 24 wk of age (Figures 7.4 and 7.5). First and second principal components (PC1 and PC2) explained 57.7 and 8.4% of the variation in samples by the PLS-DA score plot (Figure 7.4 panel A) and 49.2

and 16.9% of the variation in samples using the OPLS-DA score plot (Figure 7.5 panel A). The performance scores of the PLS-DA model for the early and late onset of lay groups at 24 wk of age were accuracy = 1.0, R^2 = 0.96, and Q^2 = 0.81 (Figure 7.4 panel C). The performance scores of the OPLS-DA model for those groups were $R^2Y(cum)$ = 0.837 and $Q^2(cum)$ = 0.791 (Figure 7.5 panel B). The permutation test results for the $R^2Y(cum)$ and $Q^2(cum)$ value were 0.964 and 0.861 between the early and late onset of lay groups at 24 wk of age (Figure 7.5 panel C). The results of permutation tests revealed that the PLS-DA (Figure 7.4 panel D) and OPLS-DA (Figure 7.5 panel C) models did not have overfitting issue, and the separations between the groups were real.

7.4.6 Identification of Significant Differential Metabolites

7.4.6.1 Pullet and Hen Groups. Significant differential metabolites (Figure 7.2 panel B) were identified and ranked by VIP values (VIP > 1.0) based on the PLS-DA model (Wang et al., 2015). The VIP is a weighted sum of squares of the PLS loadings, taking into account the amount of explained Y-variation in each dimension. The differential metabolites were divided into acyl carnitine metabolites (carnitine and acetyl carnitine), phosphotidyl choline di acyl (PC36:0AA, PC40:6AE, PC36:0AE, PC38:6AA, PC38:0AA, PC40:2AA), lysophosphotidyl cholines acyl (LYSOC20:4, LYSOC18:2, LYSOC18:0, LYSOC16:0, LYSOC14:0), hydroxy sphingomyelins (18:0SM), and organic acid metabolite (citric acid).

7.4.6.2 Early and Late Onset of Lay Groups at 24 wk of Age. The differential metabolites based on the VIP scores of the PLS-DA model (Figure 7.4 panel B) for the early and late onset of lay groups at 24 wk of age were divided into acyl carnitine metabolites (C4OH = malonyl carnitine, C16:20H = Hydroxy hexadecadienoyl carnitine, C6 = acyl carnitine), phosphotidyl choline di acyl metabolites (PC38:6AA, PC36:0AA, PC40:6AE, PC36:0AE,

PC36:6AA, PC40:2AA), lysophosphotidyl cholines acyl metabolites (LYSOC18:0, LYSOC14:0, LYSOC16:0, LYSOC18:2, LYSOC17:0), and amino acid derived metabolites (betaine).

7.4.7 Acquisition of Specific Potential Biomarkers by Receiver Operating Characteristic Curve Analysis

7.4.7.1 Pullet and Hen Groups. The aim of the multivariate exploratory ROC curve analysis was to evaluate the performance of biomarker models created through automated important compound identification. In this study, 15 significantly differential metabolites were chosen as candidate biomarkers of the pullet to hen transition (Figure 7.6 panel A). The ROC curve analysis was performed for the metabolites to clarify and estimate the maturity identification performance of the candidate biomarkers and screen potential biomarkers. A total of 13 out of 15 differential candidate biomarkers possessed an AUC more than 0.90, indicating an excellent discriminatory ability. The results indicated that acetyl carnitine, carnitine, LYSOC20:4, PC36:0AA, LYSOC18:2, LYSOC18:0, PC40:6AE, LYSOC16:0, citric acid, PC36:0AE, PC38:6AA, PC38:0AA, and LYSOC14:0 are potential biomarkers for detecting the pullet to hen transition in broiler breeders.

7.4.7.2 Early and Late Onset of Lay Groups at 24 wk of Age. A total of 15 significantly differential metabolites were chosen as the candidate biomarkers of onset of lay. The ROC curve analysis revealed that all the 15 differential metabolites had an AUC more than 0.90, representing an excellent discriminatory ability (Figure 7.6 panel B). The current study found the following metabolites as the potential biomarkers of the onset of lay in broiler breeders: acyl carnitine (C6), PC36:0AA, PC38:6AA, LYSOC18:0, PC36:0AE, PC36:6AA, malonyl carnitine, LYSOC14:0, LYSOC16:0, hydroxy hexadecadienoyl carnitine (C16:20H), LYSOC18:2, PC40:6AA, LYSOC17:0, betaine, and PC40:2AA.

7.4.8 Metabolic Pathway Analysis

7.4.8.1 Pullet and Hen Groups. According to the significant differential metabolites in the current study, metabolomics pathway analysis was constructed to further investigate the change in metabolic pathways affected by pullet to hen transition. The analysis showed that 3 pathways had the greatest significance (Figure 7.7): glycerophospholipid metabolism, citrate cycle (Tricarboxylic acid; **TCA** cycle), and glyoxylate and dicarboxylate metabolism. Changes in these pathways might be potential targets for the pullet to hen transition in broiler breeders.

7.4.8.2 Early and Late Onset of Lay Groups at 24 wk of Age. The metabolic pathway analysis for the differential metabolites between the early and late onset of lay groups at 24 wk of age revealed 2 most significance pathways (Figure 7.8): glycerophospholipid metabolism and Glycine, Serine, and Threonine metabolism.

7.5 Discussion

Body weight, body composition, physiological, and metabolic thresholds must be reached in order for sexual maturation of broiler breeder hens. Major sites involved in attaining sexual maturity include the HPG axis (maturation), liver (by formation of yolk lipids through lipogenesis), ovary (through folliculogenesis and steroidogenesis), adipose tissue (through the effect of leptin and adiponectin on HPG axis), somatotrophs (through the effects of growth hormone on insulin-like growth factor-I and the HPG axis), and thyroid axis (through modulation of effects of gonadotropins on ovarian function).

A total of 104 metabolites with different concentrations between the pullet and hen groups were screened and identified. Based on the pathway analysis for the differential metabolites identified by the VIP scores of the PLS-DA model, the main metabolic pathways

associated with these differential metabolites were lipid, energy, and amino acids metabolism. The ROC curve analysis revealed that carnitine and choline metabolites could be considered as potential biomarkers of pullet to hen transition. Most of the phosphatidylcholine metabolites and carnitine metabolites were upregulated in hens whereas lyso-phosphatidylcholine metabolites, and citric acid were downregulated. Zhu et al. (2020) performed a comparative analysis of metabolites in the liver of Muscovy ducks at different egg laying stages and indicated that the glutathione and ascorbic acid abundances were downregulated, and the choline abundance was upregulated during egg laying. The metabolomic profile changes were related to the role of the liver in fat metabolism (Cieślik et al., 2011; He et al., 2014) and yolk precursor synthesis (Wood et al., 2021). The TCA cycle is the core centre of energy metabolism. Citric acid is an important intermediate metabolite of the TCA cycle. The decrease in plasma citric acid content reflected the inhibition of glycolysis in a hamster hyperlipidemia model (Jiang et al., 2013). The authors indicated that as liver lipid content increased, the levels of TCA cycle intermediates, including plasma citrate and succinate, decreased in hamsters. In the current study, downregulation of citric acid in the hens might be due to increase in lipogenesis in the liver (Pearce, 1971) at cost of reduced glycolysis, which could be reflected by reduction in plasma citric acid. Furthermore, the increase in carnitine metabolites (e.g., acetyl carnitine) suggests mitochondrial oxidation of fatty acids, especially in the liver. Carnitine is required for the transport of fatty acids through the inner mitochondrial membrane where fatty acid oxidation takes place (Jia et al, 2014). Thus, upregulation of carnitine metabolites in the current study might be associated with the increased lipogenesis in the liver during the pullet to hen transition phase.

A total of 87 metabolites with different concentrations between the early and late onset of lay groups at 24 wk of age were identified. Pathway analysis of the differential metabolites

identified based on the VIP scores of the PLS-DA model suggested that the main pathways involved in differentiation of the early- and late-onset of lay groups at 24 wk of age were related to lipid and amino acids metabolism. The ROC curve analysis showed that carnitine metabolites, choline metabolites, and betaine could be used as potential biomarkers to predict the timing of onset of lay in broiler breeders. Phosphatidylcholine metabolites were upregulated in the early onset of lay group at 24 wk of age whereas lyso-phosphatidylcholine metabolites, carnitine metabolites, and betaine were downregulated. In the early onset of lay group at 24 wk of age, betaine might have been used for the synthesis of phospholipids or phosphatidylcholine (Eklund et al., 2005). Phosphatidylcholine is a glycerophospholipid and a principal component of the plasma VLDL monolayer. Production of phosphatidylcholine metabolites might have been upregulated to be used as the precursors of egg yolk when a hen matures and commences egg laying. Cui et al. (2020) demonstrated that as Rohman layer pullets approached sexual maturity from 125 d onward, reproductive hormonal changes (mainly estrogen) directly increased the expression of genes related to lipogenesis (fatty acid synthase) and yolk precursor (very low density apolipoprotein-II and vitellogenin-II) synthesis, which increased serum concentration of phospholipid, triacylglycerol, vitellogenin, very low density lipoprotein y (VLDLy), lecithin, total cholesterol, and triglyceride.

In the current study, increasing prepubertal and pubertal BW gains by more than 15% of the breeder-recommended target BW triggered fat metabolism and yolk precursors synthesis, which consequently advanced sexual maturity. In conclusion, this study indicated that metabolic transition during the onset of lay in broiler breeders is accompanied by certain metabolic signatures that can be used to predict the metabolic status linked to the bird's maturity. More

research is warranted to investigate the complex interactions of all the differential metabolites and reproductive axis (HPG axis) in maturing broiler breeders.

7.6 Acknowledgments

Funding from Alberta Agriculture and Forestry (Edmonton, AB, Canada), Poultry Innovation Partnership (Edmonton, AB, Canada), scholarship support of Alberta Innovates and Alberta Advanced Education, technical assistance of The Metabolomics Innovation Centre, University of Alberta (Edmonton, AB, Canada), technical assistance from Chris Ouellette and in-kind support for the precision feeding system from Xanantec Technologies, Inc. (Edmonton, AB, Canada) is gratefully acknowledged.

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7.8 Tables

Table 7. 1. Important compounds identified by fold change (FC) analysis and volcano plot[1] between pullet and hen groups[2].

Compounds	FC	log2(FC)	*P* value	Hen/Pullet[3]
LYSOC18:0	0.205	-2.280	< 0.001	Down
Trimethylamine N-oxide	0.305	-1.708	0.001	Down
Citric acid	0.320	-1.640	< 0.001	Down
Acetyl carnitine	2.844	1.508	< 0.001	Up
LYSOC17:0	0.370	-1.431	< 0.001	Down
PC38:6AA	2.684	1.424	< 0.001	Up
LYSOC14:0	0.400	-1.321	< 0.001	Down
LYSOC18:2	0.417	-1.261	< 0.001	Down
Creatine	2.277	1.187	0.002	Up
LYSOC16:0	0.440	-1.183	< 0.001	Down
LYSOC20:4	0.445	-1.167	< 0.001	Down
Taurine	0.471	-1.085	< 0.001	Down
PC36:0AA	2.052	1.037	< 0.001	Up
Trans-hydroxyproline	0.490	-1.027	0.001	Down
PC40:6AA	2.034	1.024	< 0.001	Up

[1]Volcano plot analysis is a combination of the fold change and t-test analyses, which has provided the *P* values in the table.
[2]The candidate birds for metabolomics assays were categorized as either a pullet or a hen at each blood sampling age, depending on whether they had laid an egg prior to the collection of the plasma sample.
[3]Hen/Pullet: Change in the hen group compared to the pullet group.

Table 7. 2. Important compounds identified by t-test analysis between pullet and hen groups[1].

Compounds	FDR[2]	*P* value	Hen/Pullet[3]
Carnitine	< 0.001	< 0.001	Up
Acetyl carnitine	< 0.001	< 0.001	Up
LYSOC18:2	< 0.001	< 0.001	Down
PC36:0AE	< 0.001	< 0.001	Up
18:0SM	< 0.001	< 0.001	Down
PC36:0AA	< 0.001	< 0.001	Up
Citric acid	< 0.001	< 0.001	Down
LYSOC18:0	< 0.001	< 0.001	Down
LYSOC14:0	< 0.001	< 0.001	Down
PC38:6AA	< 0.001	< 0.001	Up
LYSOC20:4	< 0.001	< 0.001	Down
PC40:6AE	< 0.001	< 0.001	Up
PC38:0AA	< 0.001	< 0.001	Down
LYSOC16:0	< 0.001	< 0.001	Down
PC40:2AA	< 0.001	< 0.001	Down
PC40:6AA	< 0.001	< 0.001	Up
16:1SMOH	< 0.001	< 0.001	Down
LYSOC17:0	< 0.001	< 0.001	Down
22:2SMOH	< 0.001	< 0.001	Down
PC36:6AA	< 0.001	< 0.001	Up
14:1SMOH	< 0.001	< 0.001	Down
16:1SM	< 0.001	< 0.001	Down
22:1SMOH	< 0.001	< 0.001	Down
Hydroxy hexadecanoyl carnitine	< 0.001	< 0.001	Down
Malonyl carnitine	< 0.001	< 0.001	Down
20:2SM	< 0.001	< 0.001	Up
HPHPA	< 0.001	< 0.001	Down
Hydroxy valeryl carnitine (Methyl malonyl carnitine)	< 0.001	< 0.001	Down
Dodecanoyl carnitine	< 0.001	< 0.001	Down
Methyl glutaryl carnitine	< 0.001	< 0.001	Down
Tetra decadienoyl carnitine	< 0.001	< 0.001	Down
Dodecanedioyl carnitine	< 0.001	< 0.001	Down
Decenoyl carnitine	< 0.001	< 0.001	Down
Hexadecadienoyl carnitine	< 0.001	< 0.001	Down
Decadienoyl carnitine	< 0.001	< 0.001	Down
Glutamic acid	< 0.001	< 0.001	Down
Hydroxy hexadecenoyl carnitine	< 0.001	< 0.001	Down
Dodecenoyl carnitine	< 0.001	< 0.001	Down
Total-dimethyl Arginine	< 0.001	< 0.001	Down
LYSOC16:1	< 0.001	< 0.001	Down
p-Hydroxy hippuric acid	< 0.001	< 0.001	Down
LYSOC28:0	< 0.001	< 0.001	Down
Hexadecenoyl carnitine	< 0.001	< 0.001	Down
Carnosine	< 0.001	< 0.001	Down
Hydroxy octadecenoyl carnitine	< 0.001	< 0.001	Down
LYSOC28:1	< 0.001	< 0.001	Down
Betaine	< 0.001	< 0.001	Down
Hydroxy tetradecenoyl carnitine	< 0.001	< 0.001	Down
Homocysteine	< 0.001	< 0.001	Down
Succinic acid	< 0.001	< 0.001	Down

[1]The candidate birds for metabolomics assays were categorized as either a pullet or a hen at each blood sampling age, depending on whether they had laid an egg prior to the collection of the plasma sample.
[2]FDR: False discovery rate [3]Hen/Pullet: Change in the hen group compared to the pullet group.

Table 7. 3. Important compounds identified by fold change (FC) analysis and volcano plot[1] between the early and late onset of lay groups at 24 wk of age.

Compounds	FC	log2(FC)	*P* value	Early/Late[2]
PC38:6AA	2.766	1.467	< 0.001	Up
LYSOC18:0	0.230	-2.114	< 0.001	Down
PC36:6AA	2.071	1.050	< 0.001	Up
LYSOC14:0	0.411	-1.281	< 0.001	Down
LYSOC16:0	0.463	-1.108	< 0.001	Down
LYSOC18:2	0.431	-1.212	0.001	Down
LYSOC17:0	0.417	-1.261	0.001	Down
Betaine	0.494	-1.015	0.001	Down
LYSOC20:4	0.399	-1.324	0.001	Down
Citric acid	0.295	-1.760	0.001	Down
Trimethylamine N-oxide	0.424	-1.235	0.001	Down
Taurine	0.470	-1.089	0.011	Down
Carnosine	0.432	-1.207	0.011	Down
Corticosterone	2.855	1.513	0.016	Up
Sarcosine	0.390	-1.358	0.034	Down
Kynurenine	0.392	-1.347	0.038	Down
Trans-hydroxyproline	0.409	-1.287	0.056	Down

[1]Volcano plot analysis is a combination of the fold change and t-test analyses, which has provided the *P* values in the table.
[2]Early/Late: Change in birds having early onset of lay compared to late onset of lay group.

Table 7. 4. Important compounds identified by t-test analysis between the early and late onset of lay groups at 24 wk of age.

Compounds	FDR[1]	*P* value	Early/Late[2]
PC38:6AA	< 0.001	< 0.001	Up
C6 (Acyl carnitine)	< 0.001	< 0.001	Down
PC36:0AA	< 0.001	< 0.001	Up
LYSOC18:0	< 0.001	< 0.001	Down
PC36:0AE	< 0.001	< 0.001	Up
PC36:6AA	< 0.001	< 0.001	Up
Malonyl carnitine	< 0.001	< 0.001	Down
LYSOC14:0	< 0.001	< 0.001	Down
LYSOC16:0	< 0.001	< 0.001	Down
Hydroxy hexadecenoyl carnitine	< 0.001	< 0.001	Down
LYSOC18:2	0.001	< 0.001	Down
PC40:6AA	0.001	< 0.001	Up
LYSOC17:0	0.001	< 0.001	Down
Betaine	0.001	< 0.001	Down
PC40:2AA	0.001	< 0.001	Down
LYSOC20:4	0.001	< 0.001	Down
16:1SMOH	0.001	< 0.001	Down
Citric acid	0.001	< 0.001	Down
Hexenoyl carnitine	0.002	< 0.001	Down
18:0SM	0.002	< 0.001	Down
Hexadecadienoyl carnitine	0.002	< 0.001	Down
Methylglutaryl carnitine	0.003	< 0.001	Down
Dodecenoyl carnitine	0.003	< 0.001	Down
Glutaryl carnitine (Hydroxyhexanoyl carnitine)	0.003	< 0.001	Down
Hydroxy hexadecadienoyl carnitine	0.004	< 0.001	Down
Tetradecenoyl carnitine	0.004	< 0.001	Down
Octadecadienyl carnitine	0.004	< 0.001	Down
p-Hydroxyhippuric acid	0.004	< 0.001	Down
Hexadecenoyl carnitine	0.004	< 0.001	Down
Hydroxy valeryl carnitine (Methylmalonyl carnitine)	0.004	< 0.001	Down
Hydroxy hexadecanoyl carnitine	0.005	0.001	Down
Glutaconyl carnitine	0.005	0.001	Down
Dodecanedioyl carnitine	0.005	0.001	Down
Hippuric acid	0.005	0.001	Down
Hydroxy tetradecenoyl carnitine	0.005	0.001	Down
16:1SM	0.005	0.001	Down
Hydroxy tetradecadienoyl carnitine	0.005	0.001	Down
Butenyl carnitine	0.005	0.001	Down
Hexadecenoyl carnitine	0.005	0.001	Down
Trimethylamine N-oxide	0.005	0.001	Down
Propenoyl carnitine	0.005	0.001	Down
Octadecanoyl carnitine	0.006	0.001	Down
Tetradecadienoyl carnitine	0.006	0.002	Down
Arginine	0.006	0.002	Down
Dodecanoyl carnitine	0.006	0.002	Down
Decadienoyl carnitine	0.006	0.002	Down
14:1SMOH	0.006	0.002	Down
LYSOC26:1	0.006	0.002	Down
Nonayl carnitine	0.007	0.002	Down
22:2SMOH	0.008	0.002	Down

[1]FDR: False discovery rate

[2]Early/Late: Change in birds having early onset of lay compared to late onset of lay group.

7.9 Figures

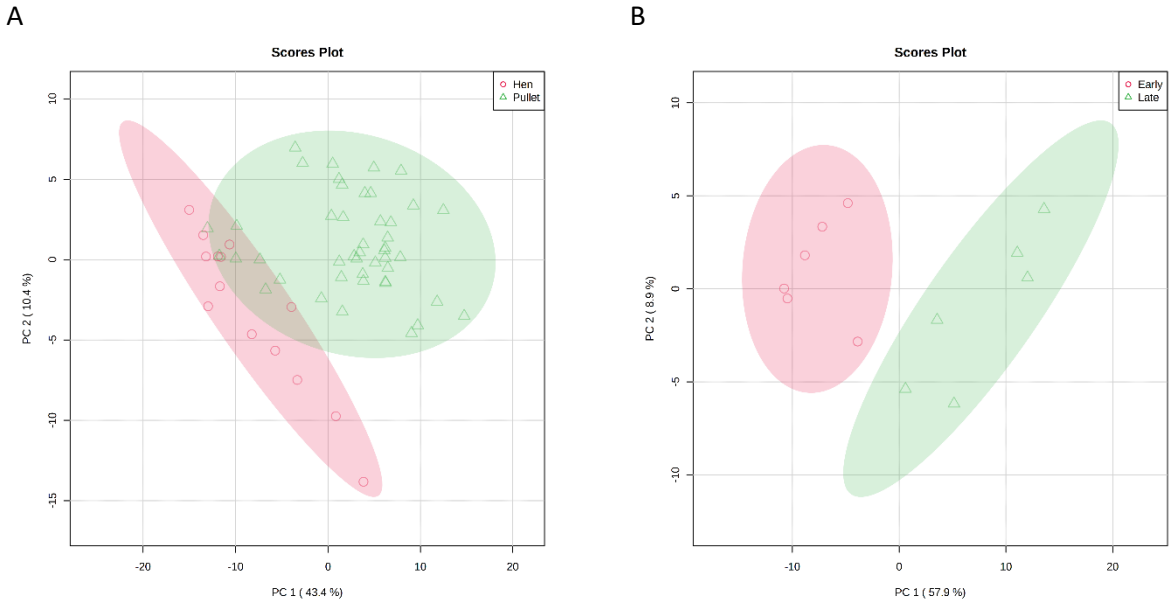


Figure 7. 1. Principal component (PC) analysis of plasma metabolomics data at 24 wk of age shows separation of metabolomes of the pullet and hen groups (A) and the early and late onset of lay groups (B).

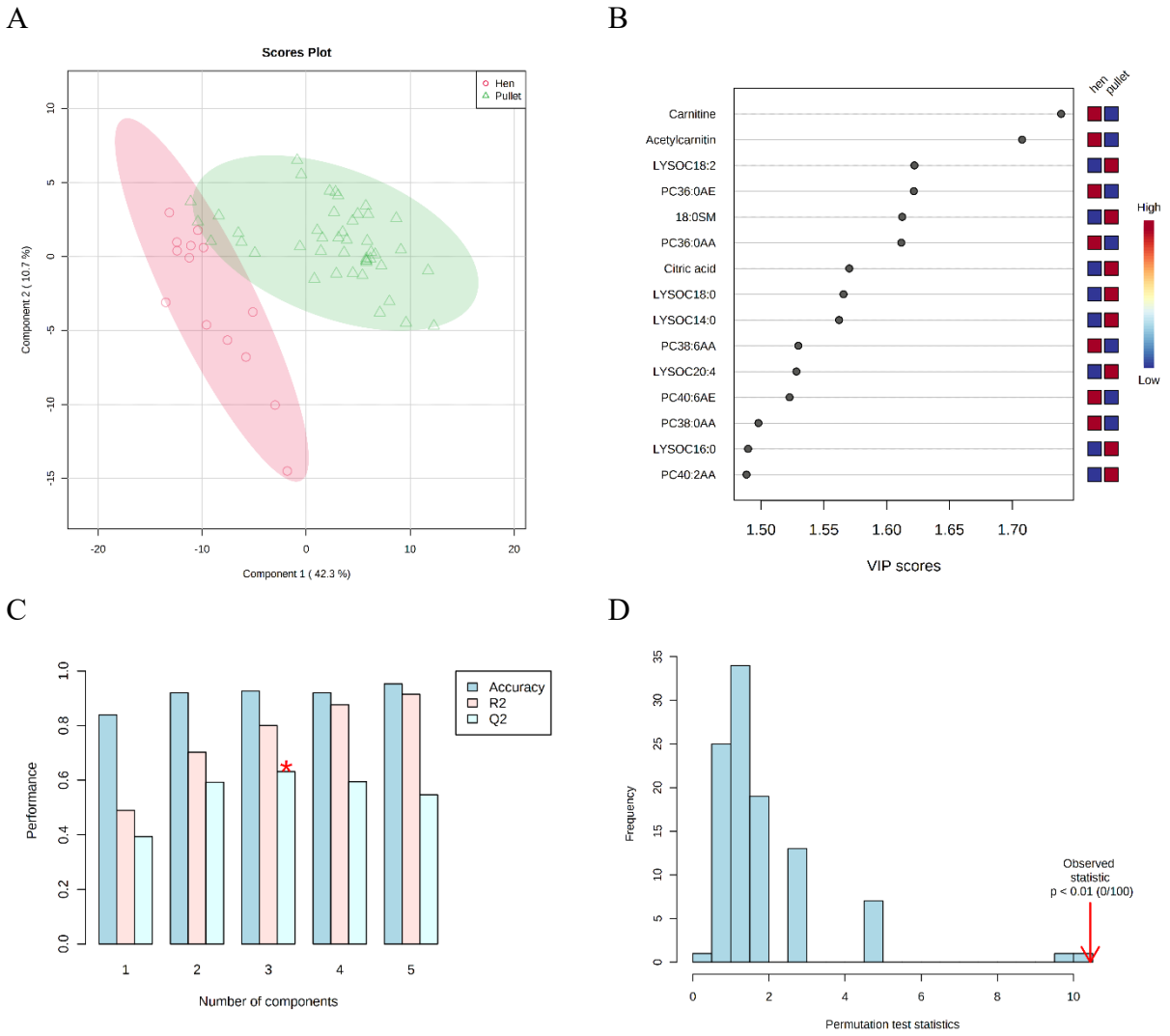


Figure 7. 2. Partial Least-Squares Discriminant Analysis (PLS-DA) of pullet and hen plasma metabolomics data (A), variable importance in the projection (VIP) scores of the differential metabolites (B), and corresponding validation plots of the fitting and predictive performance of the model (C) and the permutation test (100 times, D) of the PLS-DA model.

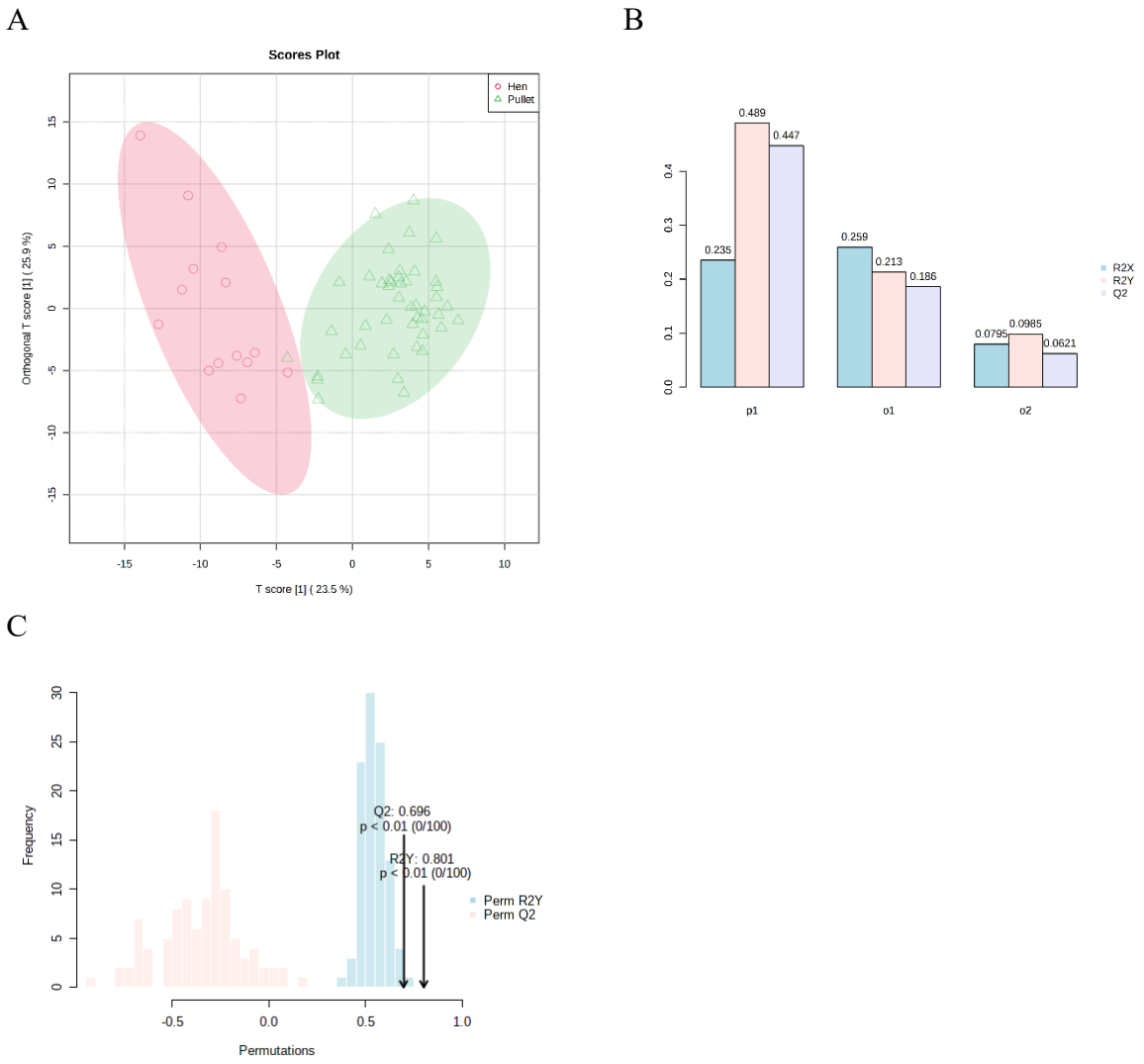


Figure 7. 3. Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) of pullet and hen plasma metabolomics data (A), and corresponding validation plots of the fitting and predictive performance of the model (B) and the permutation test (100 times, C) of the OPLS-DA model.

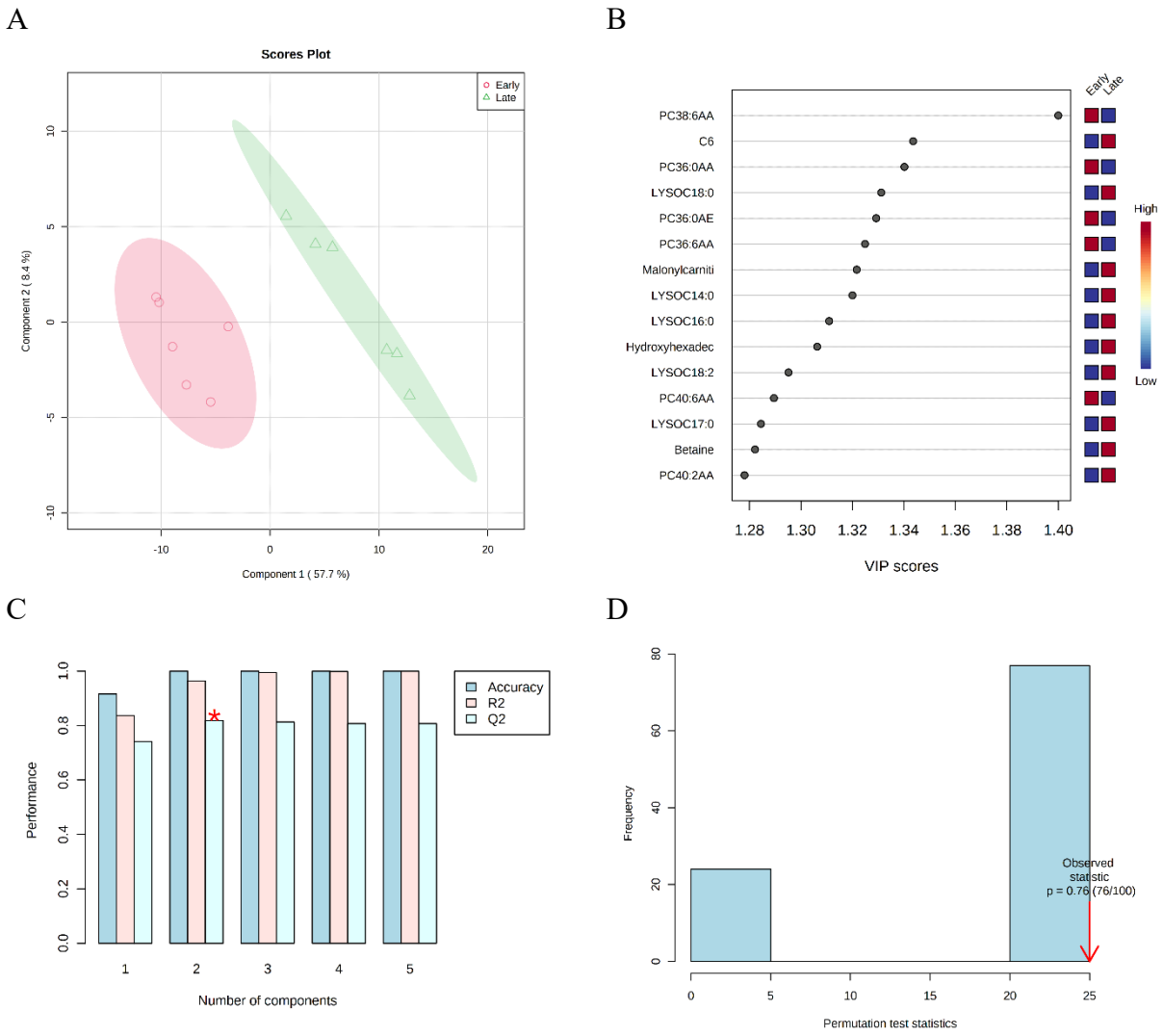


Figure 7. 4. Partial Least-Squares Discriminant Analysis (PLS-DA) of plasma metabolomics data at 24 wk of age in the early and late onset of lay groups (A), variable importance in the projection (VIP) scores of differential metabolites (B), and corresponding validation plots of the fitting and predictive performance of the model (C) and the permutation test (100 times, D) of the PLS-DA model.

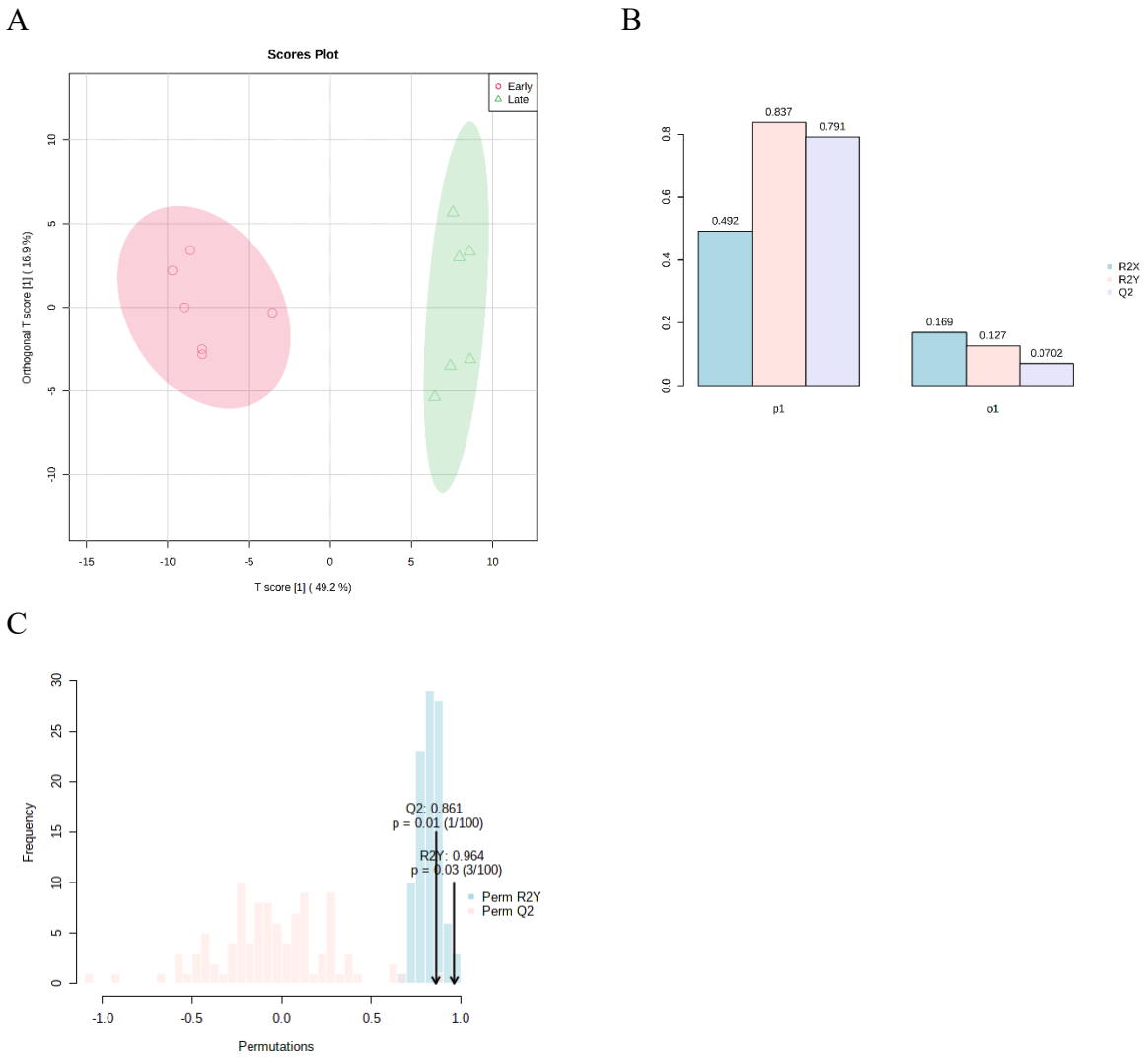
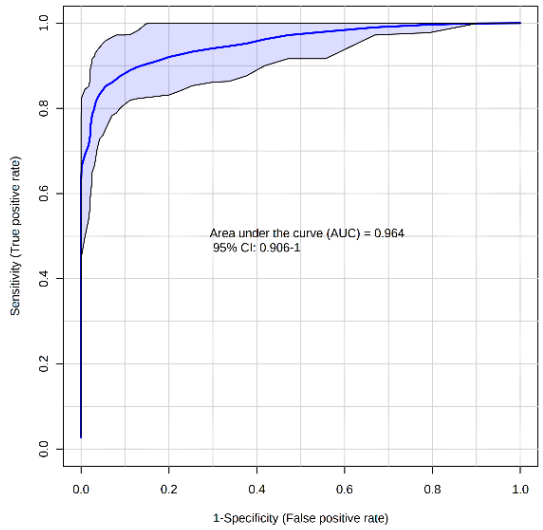


Figure 7. 5. Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) of plasma metabolomics data at 24 wk of age from birds having early or late onset of lay (A), and corresponding validation plots of the fitting and predictive performance of the model (B) and the permutation test (100 times, C) of the OPLS-DA model.

A



B

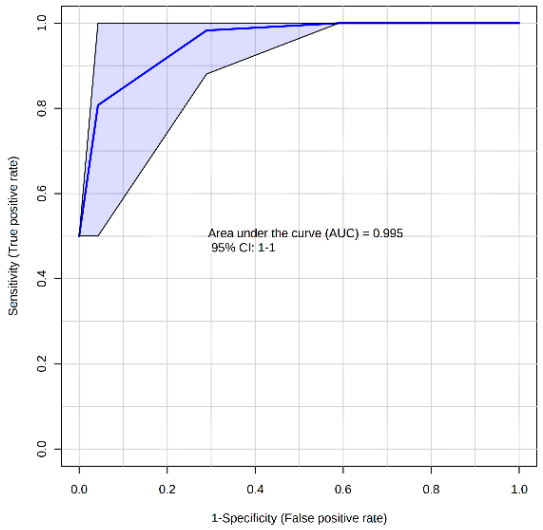


Figure 7. 6. Receiver Operator Characteristic (ROC) curve analysis to evaluate the performance of biomarker models created through automated important compound identification for top 15 differential metabolites at 24 wk of age between the pullet and hen (A) and the early and late onset of lay groups (B).

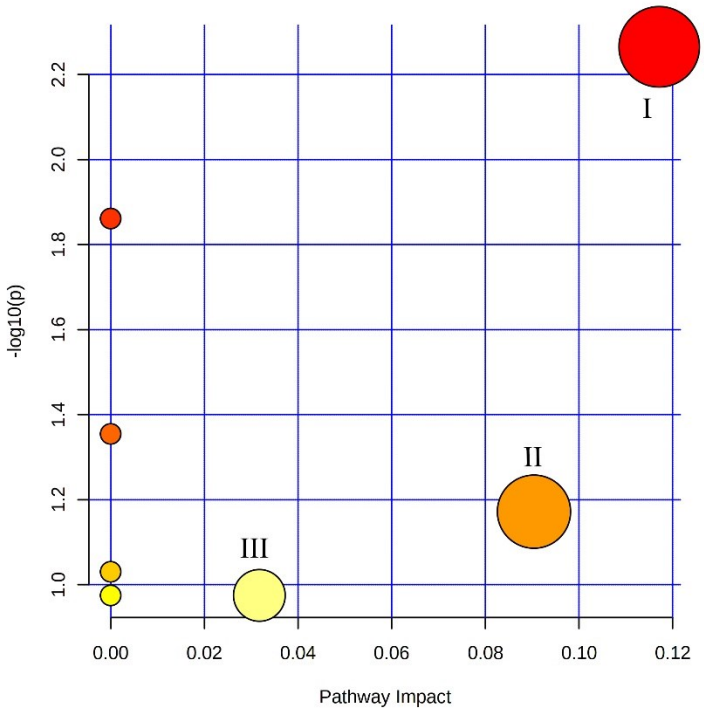


Figure 7. 7. Pathway analysis of differential metabolites between the pullet and hen groups as shown in bubble plots. Bubble size is proportional to the impact of each pathway, and bubble color represents the degree of significance, from the highest (red) to the lowest (yellow). I = Glycerophospholipid metabolism; II = Citrate cycle (TCA cycle); III = Glyoxylate and dicarboxylate metabolism.

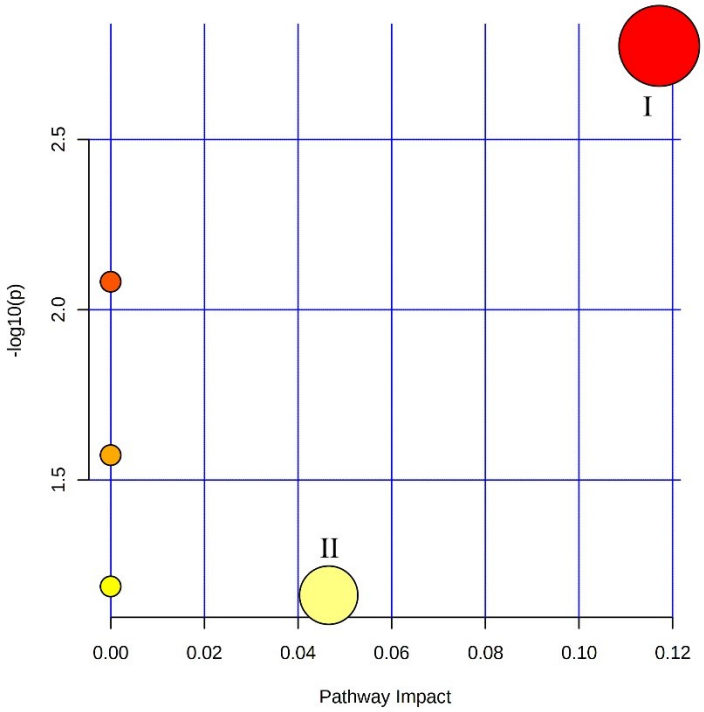


Figure 7. 8. Pathway analysis of differential metabolites between the early and late onset of lay groups at 24 wk of age as shown in bubble plots. Bubble size is proportional to the impact of each pathway, and bubble color represents the degree of significance, from the highest (red) to the lowest (yellow). I = Glycerophospholipid metabolism; II = Glycine, Serine, and Threonine metabolism.

8.0 Chapter 8: Comparison of Liquid Chromatography-Tandem Mass Spectrometry and ELISA Methods for Measurement of Plasma Corticosterone in Broiler Breeders

8.1 Abstract

Blood concentration of corticosterone (**CORT**) is a measure of stress in feed restricted broiler breeders. The RIA and ELISA have been routinely used for measuring CORT in blood, excreta, and feather. Due to the presence of some confounding factors in the aforementioned colorimetric enzyme reaction methods, some methodological difficulties have been attributed to those assays. The correlation between broiler breeder plasma CORT concentrations, measured using ELISA and a novel liquid chromatography-tandem mass spectrometry (**LC-MS/MS**) method, was the focus of the current study. A total of 36 broiler breeder pullets were used, of which 30 were randomly assigned to one of 10 unique growth trajectories, and 6 were assigned to an unrestricted group. We designed the growth trajectories using a 3-phase Gompertz growth model with 10 levels of BW gain in the prepubertal and pubertal growth phases, ranging from the breeder-recommended target BW (**CON**) to 22.5% higher (CON+22.5%), in 2.5% increments. The BW trajectories were applied to each individual bird using a precision feeding (**PF**) system, which collected BW and feed intake data. The birds were classified based on age at first egg (**AFE**), and 12 birds each having the highest and lowest AFE were selected for the CORT study. Then median photostimulation BW of the candidate birds was used to define the upper (heavy BW) and lower (standard BW) extremes, and plasma CORT levels were evaluated by ELISA and LC-MS/MS methods from their blood collected at 18, 20, 22, 24, and 26 wk of age. Concentrations of plasma CORT measured using ELISA method were highly correlated ($r = 0.95$; $P < 0.001$) with values measured using LC-MS/MS method, validating interchangeably usage of both methods to measure plasma CORT in broiler breeders. Plasma CORT levels were

not affected by photostimulation BW or breeders age, indicating same welfare status between the precision fed high and low BW groups.

Key words: broiler breeder, corticosterone, ELISA, metabolomics, feed restriction

8.2 Introduction

Numerous studies have shown that high levels of corticosterone (**CORT**) are associated with greater stress level in severely feed-restricted broiler breeders. Stressors activate the hypothalamus-pituitary-adrenocortical cascade, resulting in the release of CORT (Blas, 2015). Much effort through experimental studies has been devoted to quantifying and characterizing this association and the underlying mechanisms. In most studies, RIA and ELISA methods have been used for measuring CORT in the blood and feather (Gonzales et al., 2003; Carbajal et al., 2014; Häffelin et al., 2020; Leishman et al., 2020; Cognuck et al., 2020; Weimer et al., 2020).

ELISA assay employs a colorimetric enzyme reaction which can be confounded by many factors. The method requires the avoidance of contact with light and metal, precise control of the amount of acid added in sample analysis, and a long detection time, leading to a lack of proper validation testing (Zhong and Suo, 1998; Shu et al., 2003; Little et al., 2008; Zhou et al., 2011; Sink et al., 2008). Stanczyk et al. (2007) suggested that due to lack of standardization across steroid hormone assays (either RIA or ELISA), it is difficult to compare results across studies that use different assay platforms. Mouse plasma CORT level was measured using an ultra-fast liquid chromatography-tandem mass spectrometry (**LC-MS/MS**) method (Huan et al., 2014), but the correlation of the measures with ELISA method has remained to be elucidated. Thus, it is prudent to determine the degree in which CORT concentration measured using the classic ELISA is correlated with LC-MS/MS measure.

Broiler breeders are commonly reared by applying a substantial reduction in feed intake that controls excessive growth and maximises reproductive fitness, production persistency, and longevity (Renema and Robinson, 2004). Feed restriction programs can be categorized into quantitative (e.g. limited everyday feed restriction and skip-a-day feeding programs) and qualitative (e.g. feeding diluted diets) feed restriction programs (Carneiro et al., 2019; Zuidhof et al., 1995). Selection for increased growth rate in broilers has led to an increase in adult BW for their parent stocks (Zuidhof et al., 2014). However, broiler breeder BW targets have changed very little over the past decades (Renema et al., 2007), creating a considerable gap between growth potential of broilers and broiler breeder target BW. Over decades, reducing feed consumption to control breeder BW has increased the intensity of feed restriction, causing welfare concerns in underfed birds (van Krimpen and de Jong, 2014). The welfare aspect of different feeding programs has been assessed through physiological indices of stress such as elevated blood heterophil:lymphocyte ratio, plasma CORT content, cecal CORT content, colon CORT content, feather CORT content (Hocking et al., 2001; Mormède et al., 2007; de Jong et al., 2002; 2005; van Krimpen and de Jong, 2014; Weimer et al., 2018) and behavioural changes such as stereotypic object pecking, over-drinking, and hyperactivity (de Jong and Jones, 2006). Weimer et al. (2018) compared multiple above-mentioned stress indicators to elucidate the correlation between the measures in broiler chickens. The authors concluded that although it is prudent to measure multiple physiological indices of stress to assess the animal welfare aspect of feeding programs, blood CORT was found to be the most reliable measure of stress, showing less variation than other measures.

To reduce the intensity of feed restriction in broiler breeders, we investigated the effects of incremental increases in target BW gain, including a non-restricted group, during prepubertal

and pubertal growth phases on feeding motivation and reproductive performance (Zukiwsky et al., 2021). In the current study, we evaluated the effect of the high and low photostimulation BW on plasma CORT level as an index of welfare. The objectives of this study were to 1) determine correlation between plasma concentrations of CORT measured by ELISA and LC-MS/MS methods and 2) investigate the effects of the high and low photostimulation BW and breeder age on plasma CORT levels.

8.3 Materials and Methods

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

8.3.1 Animals and Management

The detailed experimental protocol was published previously (Zukiwsky et al., 2021). Briefly, Ross 708 pullets (n=36) were placed in a single pen containing 2 precision feeding (**PF**) stations, from hatch to 43 wk of age at a stocking density of 3.0 birds per m^2. The birds were fed commercial broiler breeder diets: starter (crumble; ME 2,726 kcal/kg, 21% CP, 1.00% Ca, and 0.45% available P) from hatch to d 34; grower (mash; ME 2,799 kcal/kg, 15% CP, 0.79% Ca, and 0.44% available P) from d 35 to d 179; and laying diet (crumble; ME 2,798 kcal/kg, 15.3% CP, 3.30% Ca, and 0.38% available P) from d 180 onward. Feed was provided through the PF system, which identified individual birds using a wing band containing a radio frequency identification (**RFID**) transponder and fed them according to how their real-time BW compared to the pre-programmed target BW (Zuidhof et al., 2019). All birds had access to the PF stations 24 hours per day throughout the experiment. The PF system provided several access to a small meal for a 60 s if the individual bird real-time BW was equal or less than its pre-programmed

target BW; otherwise, the system gently ejected the birds from the PF station. All birds had ad libitum access to water throughout the experiment.

Pullets were exposed to 8L:16D (15 lx) photoschedule during the rearing phase and were photostimulated at wk 22 by increasing the photoperiod to 11L:13D (20 lx); to 12L:12D (25 lx) on wk 23, then at wk 24 to 13L:11D (50 lx) for the remainder of the experiment. A trap nest with 8 nesting sites and a nest box with 8 nesting sites equipped with RFID readers, which identified and weighed eggs of individual hens, were installed in the room at 14 wk of age, which allowed pullets to adapt to the nesting system prior to the onset of lay.

8.3.2 Photostimulation BW and Age at First Egg

The cloacae of all hens were palpated daily to detect hard-shelled eggs in the shell gland from 20 wk onward. Presence or absence of a hard-shelled egg in the shell gland was recorded daily for each bird to determine age at first egg (**AFE**). The median BW of the multiple BW observations for individual birds at 154 d of age (22 wk) was considered as the photostimulation BW. In our previous publication, it was concluded that AFE advanced by 10.8 d per kg increase in photostimulation BW (Zukiwsky et al., 2021). Individual bird AFE ranged from 141 to 186 d of age with a median value of 175 d of age. In the current study 6 birds with lowest AFE were considered as the early onset of lay group (lower extreme of AFE), and 6 birds with highest AFE were considered as the late onset of lay group (upper extreme of AFE). The late onset of lay group included birds from the CON, CON+2.5%, CON+5%, CON+10%, and CON+12.5% treatments, and the early onset of lay group included birds from the CON+15%, CON+17.5%, CON+20%, and unrestricted treatments. The photostimulation BW of the candidate birds ranged from 2,350 g (CON group) to 4,940 g (unrestricted group) with a median value of 2,675 g. Thus, birds with a photostimulation BW greater than the median value were considered as the high BW

group and those with a photostimulation BW lower than the median value were considered as the low BW group.

8.3.3 Plasma Sample Preparation

At 18, 20, 22, 24, and 26 wk of age, blood samples (3 mL) were collected randomly from the brachial vein of all birds using a 4 mL sodium heparin vacutainer. The birds were restrained gently to minimize the stress during blood collection. The blood samples were collected 1 to 3 h after onset of photophase in the morning and were immediately centrifuged at 1,244 x g at 4°C for 15 min to recover plasma. The plasma samples were stored at –20°C until CORT measurements.

8.3.4 Experimental Design

A completely randomized controlled study was conducted to relax the Ross 708 broiler breeder-recommended growth trajectory. We fitted a 3-phase Gompertz growth model to the breeder-recommended target BW (Aviagen, 2016) to estimate the phase-specific BW gain coefficients in prepubertal, pubertal, and post-pubertal growth phases. The model had the form (Zuidhof, 2020):

$$BW_t = \sum_{i=1}^{i=3} g_i exp^{-exp^{-b_i(t-I_i)}} + \varepsilon_t$$

where BW_t was BW (kg) at time t (wk); g_i was the total amount of gain (kg) accruing in phase i ; b_i was the growth rate coefficient for the i^{th}; t was age (wk); I_i was the inflection point (wk), or the age at which growth for phase i reached its maximum rate; and ε_t was the residual error with an expected value of 0, and a normally distributed variance estimated by the software $\varepsilon_t \sim N(0,SD^2)$; i was the growth phase (i = 1 to 3) where phase 1, 2, and 3 corresponded roughly to prepubertal, pubertal, and post-pubertal growth phases, respectively. The BW gain coefficients in

the prepubertal (g_1) and pubertal (g_2) growth phases were increased from 0% (**CON**; the breeder-recommended target BW) to 22.5% higher (CON+22.5%), in 2.5% increments to create a total of 10 growth trajectories (Figure 8.1). A total of 36 broiler breeder pullets were used, in which 30 pullets were randomly assigned to one of the growth trajectories and 6 birds were assigned to an unrestricted group. The BW trajectories were applied to each individual bird using the PF system. Therefore, each bird was an experimental unit. The unrestricted group were not limited to a maximum BW and were fed every time they used the PF stations.

In the current study, birds came to lay at different ages due to being reared on various BW trajectories, creating a range for AFE criteria. We used AFE and photostimulation BW to create experimental classifications for the current CORT study. Both classification methods resulted in the same grouping. The median photostimulation BW of the candidate birds was used to define the upper (high BW) and lower (low BW) extremes. More specifically, 12 birds each having the highest and lowest photostimulation BW were selected for measurement of the plasma CORT. Thus, two experimental treatments (high vs. low BW) were compared in terms of the plasma CORT concentration.

8.3.5 Plasma Corticosterone Determination Using ELISA Method

Plasma samples were thawed on ice in the dark, and CORT concentration was determined using a CORT ELISA kit (Cayman Chemical, USA) according to the manufacturer's instructions. Briefly, the CORT ELISA standards were prepared in 9 dilutions ranging from 8.2 pg/ml to 50 ng/ml. Thawed plasma samples were vortexed and 50 µl of the samples were added in duplicate in individual wells of microtiter plates pre-coated with mouse anti-rabbit IgG antibody. Subsequently, 50 µl of CORT-acetylcholinesterase (AChE) conjugate (CORT Tracer) and 50 µl of CORT ELISA antiserum were added to the wells. Because the concentration of the

CORT tracer was held constant while the concentration of CORT in the plasma samples varied, the amount of CORT tracer that was able to bind to the CORT antiserum would be inversely proportional to the concentration of CORT in the well (sample). The antiserum-CORT (either free or tracer) complex binds to the mouse anti-rabbit IgG that has been previously attached to the well. Thereafter the plate was washed to remove any unbound reagents and then Ellman's reagent (which contains the substrate to AChE) was added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, was proportional to the amount of CORT tracer bound to the well, which was inversely proportional to the amount of free CORT in the well (originating from the plasma samples) during overnight incubation at 4°C. The inter-and intra-assay coefficients of variation were 6.2 and 10.9%, respectively.

8.3.6 Plasma Corticosterone Determination Using LC-MS/MS Assay

8.3.6.1 Sample Preparation. Plasma samples were thawed on ice, in the dark, before use. Then 100 µL of the samples (PBS as blank sample, calibration standards, quality control standards and plasma samples) was mixed with 20 µL of internal standard mixture solution and were pipetted into Eppendorf tubes. After that, 100 µL of PBS buffer was added to each tube and vortexed for 30 s. Then 1,000 µL of methyl tert-butyl ether was added to each tube for extraction. The samples were shaken for 15 min. Subsequently, samples were centrifuged at 13,000 x g and 4°C for 15 min, and 750 µL of supernatants were transferred into HPLC vials and dried under nitrogen purge until completely dry. To the dried tubes, 100 µL of derivatization solution (1.5 M Hydroxylamine in HPLC grade water) was added, followed by shaking at 150 rpm for 15 min. All the tubes were then incubated at 60°C for 1 h, and subsequently 20 µL was

injected into an UHPLC-equipped 4000 QTrap® mass spectrometer (Sciex Canada, Concord, ON) for LC-MS/MS analysis.

8.3.6.2 LC-MS/MS Method. An Agilent 1260 series UHPLC system (Agilent, Palo Alto, CA) was used for LC-MS/MS analysis with an AB Sciex 4000 QTrap® mass spectrometer. The controlling software for the LC-MS system was Analyst 1.5.2. For the HPLC work, solvent A was 0.1% formic acid in water; and solvent B was 0.1% formic acid in methanol. The gradient profile for the UHPLC solvent run was set as follows: t = 0 min, 10% B; t = 1.50 min, 10% B; t = 2.50 min, 55% B; t = 7.50 min, 95% B; t = 8.50 min, 95% B; t = 8.60 min, 10% B; and t = 12.0 min, 10% B. The flow rate was 0.5 mL/min and the sample injection volume was 20 µL. The mass spectrometer was set to a positive electrospray ionization mode with multiple reaction monitoring. The ion spray voltage was set at 5,500 V and the temperature at 550°C. The curtain gas, ion source gas 1, ion source gas 2, and collision gas were set at 40, 60, 60 and medium, respectively. The entrance potential was set at 10 V. Likewise, the decluttering potential, collision energy, collision cell exit potential, multiple reaction monitoring Q1 and Q3 were set individually for each analyte and internal standards.

8.3.7 Statistical Analysis

The Pearson correlation coefficient was calculated using the CORR procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC), to measure the strength of the linear relationship between the plasma CORT measures using the ELISA and LC-MS/MS methods. The Pearson correlation coefficient "r" ranges from –1 to 1, where r = –1 indicates a perfect negative linear relationship, and r = 1 indicates a perfect positive linear relationship. The Pearson correlation was reported as significant where $P \leq 0.05$.

Two-way analyses of variance were conducted using the MIXED procedure of SAS, with age and photostimulation BW as sources of variation. To account for correlated repeated measures, age was included in the model as a random effect, with individual birds as subjects. Pairwise differences between means were determined with the PDIFF option of the LSMEANS statement and were reported as significant when $P \leq 0.05$.

8.4 Results and Discussion

8.4.1 Correlation Between ELISA and LC-MS/MS Methods

Plasma CORT levels measured by the ELISA and LC-MS/MS methods were positively correlated (r = 0.95; $P < 0.001$). The slope (0.97) of the regression between ELISA and LC-MS/MS measures of plasma CORT indicated a high degree of agreement between the two assays (Figure 8.2). Thus, these methods can be used interchangeably to measure the plasma CORT in birds.

8.4.2 Plasma Corticosterone Concentration

There was no effect of photostimulation BW or age on the plasma concentration of CORT (Table 8.1). In our previous publication we concluded that different feeding levels among the growth treatments created a range of photostimulation BW. More specifically, from 2 to 27 wk of age, ADFI increased in a range of 5.5 to 41.5 g per kg increase in photostimulation BW (Zukiwsky et al., 2021). Some previous research indicated elevation of plasma CORT levels attributed to increased feed restriction in broiler breeders (Mormède et al., 2007; de Beer et al., 2008). Aranibar et al. (2020) compared the effect of a skip-a-day feeding program and feeding broiler breeders with soybean hulls on the off-feed day in a skip-a-day feeding program on concentration of plasma CORT. Their results showed that plasma concentration of CORT was

greater in both groups 48 h after consuming the on-day feed amount compared to that of measured at 24 h after feeding. The authors concluded that the degree of feed restriction and the length of the fasting period between feedings had the most influence on plasma CORT level. Food restriction or starvation increased the mean glucocorticoids levels in humans and rat (Garcia-Belenguer et al., 1993; Kenny et al., 2014). Recently Manu et al. (2020) investigated saliva cortisol responses to feeding frequency in pregnant sows under isocaloric intake. The authors concluded that as all treatments groups had similar energy intake per kg live metabolic BW, splitting the limited feed from 1 meal into 2 or 3 meals and fed multiple times within the day did not alter the basal cortisol concentrations in pigs. Although in the current study the birds were raised under different degrees of growth restriction programs, the individuals daily feeding frequency was greater than the conventional one-time meal per day. The results of daily meal frequency have been shown in our previous article (Zukiwsky et al., 2021). Briefly, the meal frequency for the restricted and unrestricted groups increased over a range of 1.8 to 5.8 meals per kg increase in photostimulation BW from 2 to 37 wk of age. Therefore, it can be concluded that the PF system provided frequent meals per day, so that the length of fasting between meals was not long enough to affect the plasma CORT level.

To alleviate the intensity of feed restriction in broiler breeders, various degrees of relaxed growth targets were applied on pullets using the PF system. No effects of photostimulation BW or breeder age were observed on plasma CORT concentration as an index of animal welfare. The results of the current study indicated highly positive correlation between CORT measures using ELISA and LC-MS/MS methods. This means that both methods can be used accordingly to measure plasma CORT. Investigation of diurnal rhythm in plasma CORT levels warrants further study.

8.5 Acknowledgments

Funding from Alberta Agriculture and Forestry (Edmonton, AB, Canada), Poultry Innovation Partnership (Edmonton, AB, Canada), scholarship support of Alberta Innovates and Alberta Advanced Education, technical assistance of The Metabolomics Innovation Centre, University of Alberta (Edmonton, AB, Canada), technical assistance from Chris Ouellette and in-kind support for the precision feeding system from Xanantec Technologies, Inc. (Edmonton, AB, Canada) is gratefully acknowledged.

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8.7 Tables

Table 8. 1. Plasma concentration of corticosterone (ng/ml) measured by ELISA and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods in heavy and light broiler breeders[1].

	ELISA				LC-MS/MS			
	Heavy BW		Light BW		Heavy BW		Light BW	
Age	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
18	1.46	0.61	2.40	0.97	1.50	0.66	2.20	0.76
20	0.73	0.38	0.49	0.18	0.72	0.45	0.50	0.20
22	2.03	0.70	1.21	0.44	2.08	0.78	1.18	0.54
24	1.39	0.38	0.58	0.21	1.47	0.35	0.49	0.14
26	1.26	0.44	1.06	0.23	1.23	0.46	1.06	0.16
Sources of variation	*P*-value							
Age	0.14				0.20			
BW	0.49				0.33			
Age x BW	0.64				0.49			

[1]The median photostimulation BW of the candidate birds for measuring plasma corticosterone (CORT) concentration was used to define the upper (the high BW group) and lower (the low BW group) extremes.

8.8 Figures

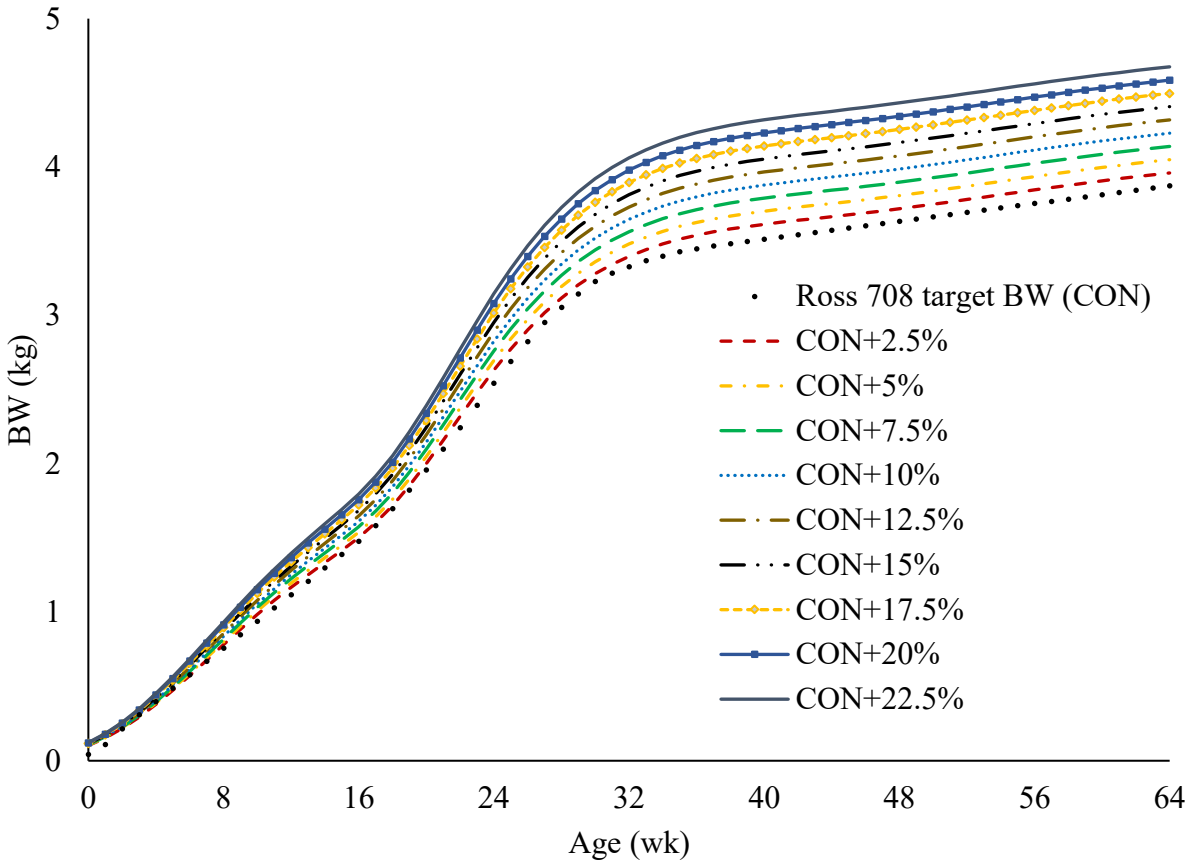

Figure 8. 1. Growth trajectories designed using estimated coefficients of a 3 phase Gompertz model. General model form was $BW_t = \sum_{i=1}^{i=3} g_i exp^{-exp^{-b_i(t-I_i)}}$ where BW_t was BW (kg) at time t (wk); i was the growth phase (i = 1 to 3) where phase 1, 2, and 3 corresponded roughly to prepubertal, pubertal, and post-pubertal growth phases, respectively, g_i was the total amount of gain (kg) in phase i ; b_i was the growth rate coefficient; t was age (wk); I_i was the inflection point (wk), or the age at which growth for phase i reached its maximum rate. The model was fitted to the Ross 708 breeder-recommended target BW to estimate the phase-specific BW gain coefficients in prepubertal, pubertal, and post-pubertal growth phases. The BW gain coefficients in the prepubertal (g_1) and pubertal (g_2) growth phases were increased from 0% (CON) to 22.5% (CON+22.5%), in 2.5% increments to create 10 unique growth trajectories.

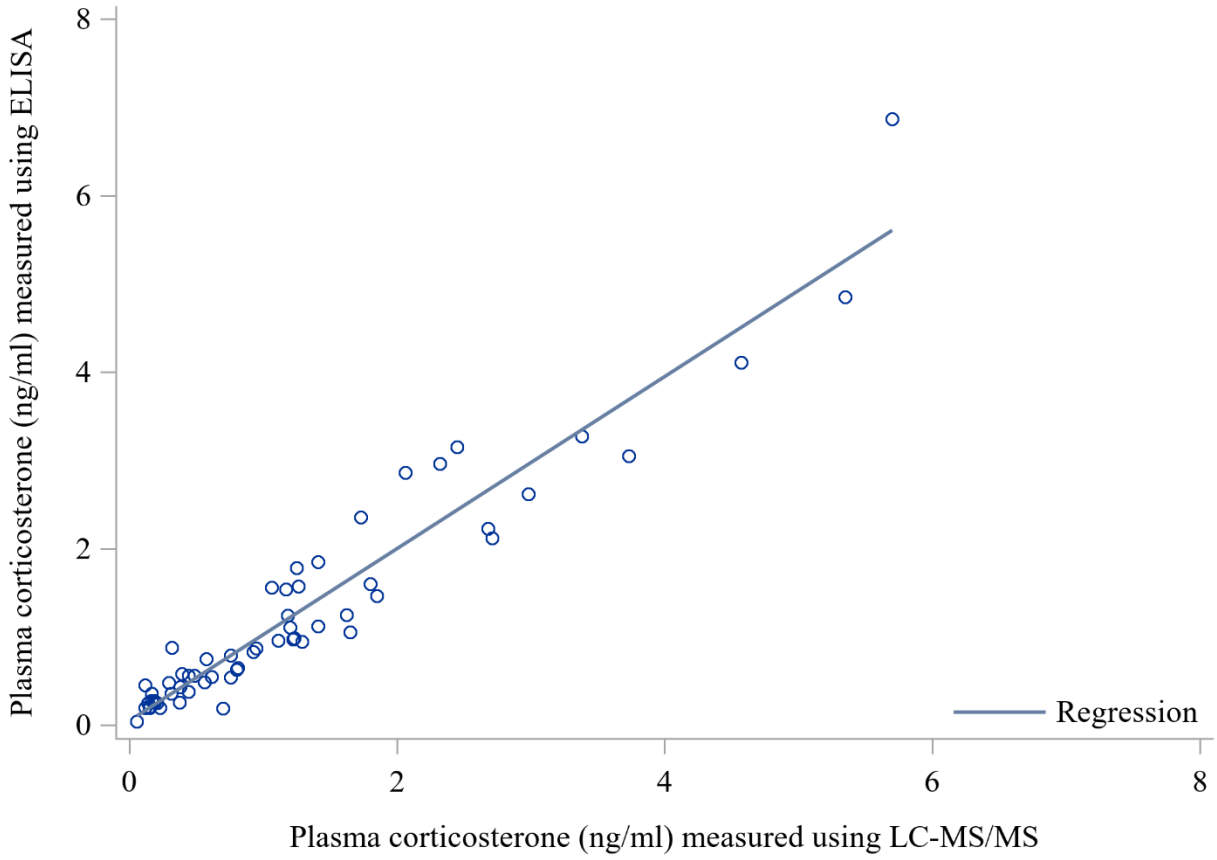

Figure 8. 2. Linear relationship between plasma corticosterone (CORT) measures using ELISA and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods in heavy and light BW broiler breeders. Regression equation was: Plasma CORT (ng/ml) measured using ELISA = 0.05 + 0.97 Plasma corticosterone (ng/ml) measured using LC-MS/MS. $R^2 = 0.91$ and Pearson correlation coefficient = 0.95.

9.0 Chapter 9. Synthesis

In the current chapter, I will elaborate the theme of my thesis, trying to answer the following questions. What was the main research question that led us to design the current study? How does the current study differ from what has been done before? What was novel in the current research? What new relationships have I discovered? What is the impact of the current research on society, science, and poultry industry? What can be recommended for future studies?

9.1 Theme of the Current Thesis

The central theme of the current thesis was to evaluate new growth strategies to circumvent severe feed restriction in broiler breeders raised under the current breeder-recommended BW target. Recent studies suggest that the intensity of feed restriction level has increased in commercial broiler breeders, which raises poor reproductive performance and welfare concerns. More specifically, some underfed modern broiler breeders do not have enough body fat reserves to commence sexual maturity (van Emous et al., 2015; van der Klein et al., 2018a,b; Zuidhof, 2018). In addition, severe feed restriction has raised welfare concerns (Mench, 2002, van Krimpen and de Jong, 2014; D'Eath et al., 2009; Tolkamp and D'Eath, 2016). Therefore, we developed the following research questions. How can relaxing growth restriction and concomitant increases in feed intake affect sexual maturity and reproductive performance of broiler breeders? What are the intergenerational effects of relaxed maternal growth restriction in broiler breeders? To answer these questions, we designed increased growth trajectories compared to the breeder-recommended target BW. In an attempt to increase fitting and predictive performance of the Gompertz growth model and to investigate the effect of minor feed restriction on energy efficiency, a pilot study was done using two heritage chicken lines (New Hampshire and Brown Leghorn). The heritage lines are traditional forms of modern breeders. The genetics

that the poultry industry uses has changed compared to local genotypes; however, the whole system works in a similar way. Heritage chickens are important for breeders and industry to protect valuable genes and traits over the long term. Preserving local breeds allows conserving the traits that could aid adaptation to future environmental and production conditions. Therefore, it is important to conduct research on heritage chickens to preserve local genetics. We used a precision feeding (**PF**) system to feed heritage chickens, broiler breeders, and progeny broiler chickens individually. Using the PF system provides an opportunity to collect big data to run mathematical models to study relationships among the pieces of the whole biological system. The BW and feed intake data collected by the PF system were used to model growth data (Chapter 3) and develop novel energy partitioning models (Chapter 5).

9.2 Unique Approaches

We used a relaxed feed restriction approach to reduce the intensity of feed restriction in broiler breeders. We used a triphasic Gompertz model with its biologically relevant continuous parameters (total amount of gain, growth rate, inflection point or the age at which growth for each phase reaches its maximum rate) to design strategic growth trajectories. Relaxing growth restriction was done through manipulating growth parameters in the prepubertal and pubertal growth phases to develop unique growth trajectories, which provided a systematic evaluation on the effects of the growth patterns. More specifically, we increased total gain in prepubertal phase by 10% and advanced timing of pubertal growth by 5, 10, 15, and 20% compared to the Ross 708 breeder-recommended target growth. This was a strategic approach because it was hypothesized that the amount of gain in the prepubertal phase would allow the birds to build a robust foundation (e.g. increase in shank length). Achieving adequate body frame development threshold provides the bird the foundation for a successful laying cycle (Shi et al., 2020).

Likewise, earlier pubertal growth could let the body accumulate enough body reserves (e.g., fat) for sexual maturation. The current approach was different compared to previous research where discrete BW targets were used. For instance, previous studies increased 20 wk target BW by 8% (Fattori et al., 1991; van Emous et al., 2013), 16% (Gous and Cherry, 2004), 20% (Ekmay et al., 2012), 21% (Renema et al., 2001a,b), 13% (Sun and Coon, 2005). Other studies increased target BW by 20% at 18 wk of age (Hocking et al., 2002) and 22% at 21 wk of age (van der Klein et al., 2018a,b). In the current thesis however, we used continuous growth parameters to design and evaluate growth trajectories. Using analysis of covariance (**ANCOVA**), we evaluated the effects of manipulating the growth parameters systematically, which is important for defining optimal growth curves.

As we evolve as a society, one of the goals in animal research is to maximize the value of our research while reducing the number of animals required. Another main difference between the current study and others was in the experimental design. The experimental design in this thesis was a leading example of innovation in experimental design with the goal of reducing the number of birds required for research. More specifically, this study was designed as a randomized controlled study but not as a traditional factorial design. In fact, using ANCOVA, which is a combination of analysis of variance (**ANOVA**) and regression analysis, provided more statistical power compared to a traditional ANOVA in a factorial design study. If the design of the study were a factorial design, using the pubertal inflection (I_2) as a factor would have resulted in a degree of freedom (df) of 4 for that factor (5 levels of $I_2 - 1 = 4$). This df would have been subtracted from the total df to obtain the residual df (df_{error}). We used ANCOVA analysis where df for the I_2 (as a covariate) was just 1. This df is less than that of in the ANOVA scenario which was already explained (df=4). In fact, we are saving 3 df using

ANCOVA instead of a traditional ANOVA. Now considering the MS_{error} formula as (SS_{error}/df_{error}), the ANCOVA method results in a smaller MS_{error} compared to the ANOVA analysis. This increases power of the analysis in ANCOVA (using both ANOVA and regression) compared to ANOVA. Using 4 birds per treatment (growth trajectory) in a completely randomized study analysed using ANCOVA, was adequate in this study. However, I acknowledge that this was a pilot study for a larger study where more replicates will be used to increase reliability of measurements. The next study will be conducted in Dr. Zuidhof's lab at the University of Alberta.

In Chapter 3, we increased fitting and predictive performance of the traditional Gompertz growth model by including random terms associated with different sources of unexplained variation. Although several nonlinear mixed models have been used to model growth data (Aggrey, 2009; Karaman et al., 2013; Schinckel et al., 2005), the effect of including random terms associated with different sources of unexplained variation on the estimation accuracy of growth parameters has not been thoroughly investigated, and to our knowledge are relatively new to the poultry science literature. Including random terms associated with different sources of unexplained variation in a modeling procedure can improve inferential efficiency.

The experiments described in Chapters 4, 5, and 6 were the first studies in which the effects of a continuous growth parameter (pubertal growth inflection time) were investigated rather than a discrete variable. More specifically, the effects of timing of pubertal growth phase were investigated on reproductive performance of breeders (Chapter 4), energy efficiency (Chapter 5), and on progeny growth performance (Chapter 6). As discussed earlier, most previous studies tried to relax growth restriction in broiler breeders using discrete growth targets. Using a continuous growth parameter in the current study was a preliminary step for

optimization practices. Continuous parameters can be used to design hypothesis-based BW trajectories for optimization purposes. Future studies can be designed to optimize broiler breeder growth trajectories using a response surface method taking into account multiple response criteria.

Energy requirement predicting models have been used to establish optimized levels of dietary nutrients and more profitable feeding programs for poultry (Sakomura, 2004), yet no comprehensive work was dedicated to investigating the effects of a chunking approach on ME partitioning model bias, fitting, and predictive performance. In Chapter 5, a chunking procedure was applied on the PF system data to increase robustness of broiler breeder energy partitioning models and to improve inferential efficiency. Data chunking procedure was done through grouping BW and feed intake data into different period lengths (daily, 4-d, weekly, 2-wk, and 3-wk). To our knowledge, this is the first systematic investigation of the effects of chunking approach on ME partitioning models bias, fitting, and predictive performance. Chunking BW and feed intake data into 3-wk periods provided the most parsimonious energy partitioning model based on lower autocorrelation bias, closer fit of the estimates to the actual data (lower model MSE and R^2 closer to 1) compared to the models derived using other chunk sizes.

The effect of including random terms associated with different model parameters (individual maintenance ME and age) on the fitting performance of the models has been investigated (van der Klein et al., 2020), yet it is not clear how inclusion of different random terms could bias the predictive performance of ME intake partitioning models. In Chapter 5, we evaluated predictive performance of 20 models using a 5-fold cross validation method. A mixed effect ME partitioning model containing a random term associated with individual maintenance

requirement in a 3-wk chunked data provided the greatest predictive performance among the models that were evaluated.

Recently, new advances in analytical chemistry techniques have allowed scientists to simultaneously identify and quantify numerous metabolites within a single cell, tissue, or biofluid. Poultry blood metabolome has been analysed to investigate the effects of feed restriction in Cob 500 broilers (Metzler-Zebeli et al., 2019); selection for 16-wk BW in turkeys (Clark et al., 2019); and stocking density in geese (Ying et al., 2021). However, no research was found that investigated broiler breeder plasma metabolome profiles. Chapter 7 introduced a first investigation on potential biomarkers for predicting sexual development in broiler breeders through analysing the plasma metabolome profile. One can argue that observing an egg (first egg) on a farm would be the easiest way to determine sexual maturity in breeders. That being said, we discovered some key differences in metabolic biomarkers that give clues to the physiological and metabolic shifts resulting from sexual maturation. The ultimate goal is to design a point of care device (similar to a portable blood glucometer) to measure broiler breeder plasma metabolome in real time at the flock level and in a matter of seconds. Then, the poultry industry can use the extracted data to evaluate sexual development status in a flock.

Chapter 8 of the current thesis determined correlation between plasma concentrations of corticosterone (**CORT**) measured by ELISA and LC-MS/MS methods for the first time. Mouse plasma CORT level was measured using an ultra fast liquid chromatography-tandem mass spectrometry (**LC-MS/MS**) method (Huan et al., 2014), yet the correlation of the measures by ELISA and LC-MS/MS methods had not been elucidated. Thus, it was important to determine the degree in which CORT concentration measured using the classic ELISA is correlated with LC-MS/MS measure to validate different assays. Compared to ELISA, LC-MS/MS is a faster

method that is highly reliable and has excellent sensitivity and specificity to detect very low levels of CORT (Huan et al., 2014). The process is almost completely automated, with few manual steps. When this is coupled to high precision instruments the results are highly reproducible with low coefficient of variance. One can argue that using a standard solution containing a known level of CORT can be used to validate different assays. This can be true for examining the accuracy of an assay, but the precision should not be taken for granted in validation procedures. Lack of precision in an assay (e.g. colorimetric assays) lead to high variability in the reported plasma CORT levels in the literature (Stanczyk et al., 2007; Zhou et al., 2011; Scanes, 2016).

9.4 New Hypotheses Developed in the Current Thesis

As achieving a critical threshold of body composition and fat during the juvenile stage is required to support the demands for egg formation throughout a laying cycle, pullet body fat percentage was estimated in Chapter 4. It was shown that carcass fat at sexual maturity is between 11-15% of total BW (Joseph et al., 2000; Renema et al., 2007). The estimated body fat in the current study was 8.04 ± 0.38 and 8.47 ± 0.38% for the birds with standard and 10% higher prepubertal gain, respectively. All birds reached sexual maturity and commenced egg laying; thus, we conclude that the minimum body fat threshold for sexual maturation is below 8%. In addition, a new definition for feeding motivation was developed in Chapter 4. Previously, feeding motivation index was evaluated using daily station visit frequency (Zukiwsky et al., 2021). Visit:meal ratio indicates feed seeking motivation determined by the number of meals allowed. Thus, it could be hypothesized that visit:meal ratio might be a better indicator of feeding motivation compared to daily station visit frequency. Results presented in the current

thesis showed that using visit:meal ratio could capture intergroup differences better than daily station visit frequency on its own.

Previously published energy partitioning models with Ross 708 broiler breeders (Pishnamazi et al., 2015; van der Klein et al., 2020) along with the model developed by the current thesis (Chapter 5) estimated a lower energy requirement compared to the recommended age-specific ME intake data by Ross 708 guideline. This motivated me to evaluate breeder-recommended target BW curves based on the guideline-recommended ME intake data. More specifically, we applied age-specific Ross 708 performance objectives data (Ross 708 performance objectives, 2016) into the following energy partitioning model to estimate age-specific ADG in the model:

$$MEI_d = (100.47 \times BW^{0.56}) + (3.49 \times ADG_p) + (3.16 \times ADG_n) + (2.96 \times EM)$$

where MEI_d = daily ME intake (kcal/d); BW = BW (kg); ADGp = positive ADG (g/d); ADGn = negative ADG (g/d); EM = egg mass (g/d). Then we calculated expected age-specific BW based on the estimated ADG for each age. Estimated target BW curve was higher than the breeder-recommended target BW. It means that the breeder recommended ME intake does not match the guideline-recommended target BW. More convincing reasons for increasing the current breeder-recommended target growth result from other Chapters of the current thesis. Increasing prepubertal gain and earlier pubertal growth increased reproductive performance of breeders (Chapter 4), probably by allowing pullets to approach a sufficient foundation and appropriate body fat level for sexual maturation. The strategy of earlier pubertal growth could reduce hunger in broiler breeders during both the rearing and laying phases. Chapter 8 concluded that increasing the maternal target BW increased offspring growth performance, which subsequently

increased profitability for both hatching egg producers and the supply chain as a whole (Appendices A and B).

Based on the results of Chapter 5, we developed some hypotheses regarding energy intake modeling in broiler breeders, which can be relevant to consider in other species as well. Increasing BW and feed intake data chunk size from daily to a 3-wk period decreased coefficient for metabolic BW (maintenance requirement) with concurrent increased coefficients for daily gain and EM requirements in all models. We hypothesized that a reduction in the variation of the ADG and EM due to an increased chunk size could increase their estimated coefficients in an energy partitioning model. Therefore, we recommend chunking data required for energy partitioning at least into 3-wk sizes while developing and reporting energy partitioning models in animals. It would also be valuable to evaluate the effects of chunking data into longer sizes (e.g. 4 wk) on fitting and predictive performance of energy partitioning models.

9.5 Impact of the Thesis on Science

Improving the traditional Gompertz growth model by incorporating random terms associated with individual mature BW and rate of maturing adds to the existing growth modeling science. The random coefficients obtained from the improved Gompertz growth model (Chapter 3) could be used as a tool in different scenarios of poultry production system such as stochastic prediction of BW of individuals at any age. Using a dynamic stochastic simulation model, age-specific BW of individuals can be predicted based on random variates drawn from relevant probability distributions obtained from the literature. Predicting age-specific BW is important to better match nutrient supply to nutrient requirements. Furthermore, random coefficients, provided by a non-linear mixed-effect growth model, are needed to predict, and evaluate the economic impact of management decisions on designing target growth curves, breeding

programs, and nutritional management decisions. Simulation of growth curves can be used to predict the variation in BW at each specific age or the age required for each bird to reach a particular target BW. Then, managerial decisions (e.g. growth scenarios) can be optimized by conducting an economic analysis to maximize the profit (minimizing cost and maximizing revenue).

Zuidhof (2020) discussed application of multiphasic growth models in designing hypothesis-based BW trajectories. Chapter 4 of the current thesis is a great example of a hypothesis-based approach to study and optimize growth trajectories in broiler breeders using continuous growth parameters. Chapter 4 provides a systematic evaluation of growth trajectories that compares reproductive outcomes of broiler breeders in response to changes per unit of growth parameters. Although Zukiwsky et al. (2021) conducted a systematic evaluation of growth trajectories, Chapter 4 further examined the effects of advancing (decreasing) pubertal growth phase concomitant with increasing prepubertal growth. This type of study helps fill knowledge gaps on how strategic and systematic manipulation of continuous growth parameters can affect breeder reproductive performance.

Chapter 5 explained a robust procedure of creating energy partitioning models containing valid estimated coefficients for maintenance, growth, and egg production, which adds to existing studies in two ways. Firstly, it explicitly improves accuracy in estimation of coefficients, thereby going beyond the common mathematical perspective of modelling procedures. Secondly, it increases predictive performance of ME intake models. This would allow precise estimation of energy requirements and feed intake of each bird, which can be used to match nutrient supply with nutrient requirements of individual birds.

Chapter 6 adds to the ever-growing evidence that maternal growth strategies can affect offspring performance. However, there is little data on effects of alterations of maternal prepubertal BW gain and pubertal growth timing on progeny performance in the literature. This chapter elaborates the maternal effects of strategically designed growth trajectories based on advancing the timing of the pubertal growth phase in breeders, which can be used in optimization of maternal growth trajectories. This adds to the existing science that multiple sets of responses, including progeny response, should be considered while defining optimum growth management strategies in broiler breeders.

Profiling broiler breeder plasma metabolome around sexual maturity indicated physiological and metabolic shifts during the pullet to hen transition period (Chapter 7). Identifying underlying biological mechanisms during the transition phase provides valuable insights into understanding limitation of reproduction and metabolism related reproduction issues in broiler breeders. Investigating plasma metabolome allows us to ask "What has happened and what is happening" in the body. Therefore, it could be used as a tool to evaluate ongoing managerial decisions (e.g. managing target BW). More specifically, concentration of plasma differential metabolites, involved in the pullet to hen transition, can be compared to the threshold amount to evaluate the sexual development status in a flock.

Chapter 8 determined high correlation between plasma concentrations of CORT measured by ELISA and LC-MS/MS methods, which adds to the existing science. Although the ELISA method has been used for many years to measure plasma CORT, it is not as efficient as the LC-MS/MS method in terms of running time and required sample amount. In addition, only one steroid hormone (e.g. CORT) can be measured by an ELISA kit, whereas LC-MS/MS method can quantify a wider range of steroid hormones in a single run. Furthermore, mass

spectrometry offers the highest sensitivity and precision for the identification and detection of analytes. The mass spectrometry process is almost completely automated, with few manual steps. Because this is coupled to high precision instruments the results are highly reproducible with low coefficients of variance. Being a colorimetric method, ELISA is in danger of being confounded by factors that could affect the intensity of color in the ELISA wells, reducing reproducibility of the method among different runs. Together, these advantages perhaps make the LC-MS/MS assay preferable to the ELISA method.

9.6 Impact of the Thesis on the Poultry Industry and Society

Regression equations were created based on the experimental data (Chapter 4) to predict the number of settable eggs and average daily feed intake (ADFI) of broiler breeders during the rearing and the laying phase (Table 9.1). The predicted parameters of interest were used in a partial budget model to evaluate the economics of the experimental growth trajectories in the broiler breeder sector (Appendix A). Margin over feed and pullet cost was estimated for the broiler breeder sector. The partial budget for the hatching egg sector predicted that increasing prepubertal BW gain by 10% along with advancing the pubertal growth inflection by 15 or 20% resulted in greater margin over feed and pullet cost compared to the breeder-recommended growth trajectory (scenarios 9 and 10 vs. scenario 1 in Table 9.3 and Figure 9.3). If a hatching egg producer switched from the breeder-recommended BW target scenario (scenario 1 in Table 9.3 and Figure 9.3) to scenario 9, the model-predicted margin over pullet and feed cost until 42 wk of age would increase by $0.75/hen; the increase in profitability would be $2.17/hen for scenario 10.

For the supply chain as a whole, regression equations were created based on the progeny experiment data (Chapter 6) to predict broiler 35 d BW and cumulative feed intake (Table 9.2). In the partial budget analysis for the broiler sector (Appendix B), differential chick cost estimated in

the broiler breeder partial budget (Table AA.3), broiler 35 d live BW, and cumulative feed consumption (Table AB.2) were included in the broiler margin calculation. Therefore, margin for this sector is based on margin over feed and chick cost, accounting for differences in the cost of producing the broiler chicks. The progeny chicks originated from two maternal ages (35 and 42 wk). The margin over feed and chick cost estimated for maternal scenario 1 (breeder-recommended scenario) from 35-wk old hens was used as a reference to compare the margin of other maternal growth scenarios (from both 35- and 42-wk old hens). All maternal growth scenarios increased margin over feed and chick cost compared to that of the breeder-recommended maternal growth scenario (scenario 1), except for scenario 6 from 35-wk old hens. For maternal growth scenario 6 (from 35-wk old hens), the margin over feed and chick cost was lower than that of scenario 1 by $0.0038/kg of live broiler chicken (Figure 9.2). The highest differential margin over feed and chick cost was for scenario 10, from 42-wk old hens, where the margin over feed and chick cost was greater than that of scenario 1 (from 35-wk old hens) by $0.0836/kg live chicken. As shown in Table 6.2 (Chapter 6), 35 d BW of broilers from 42-wk old hens were greater than that of broilers from 35-wk old hens (1,955 vs. 1,903 g, respectively), which increased revenue for the 42-wk old hens' offspring cohort. Therefore, the margin over feed and chick cost for the maternal growth scenarios from 42-wk old hens was greater than that of the scenarios from 35-wk old hens (Figure 9.2).

The partial budget predicted a greater margin over pullet and feed cost in growth scenario 10 compared to the current breeder-recommended BW scenario (Scenario 1), for both the hatching egg sector, and the supply chain as a whole. Because the data suggest an economic advantage, we recommend adoption of scenario 10 as an optimal strategy (Table 9.3 and Figure 9.3). Finally, as

severe feed restriction has raised social concerns about animal welfare, relaxing feed restriction would begin to address these social concerns.

9.7 Direction for Future Research

As explained earlier in this chapter, the design of the current study aimed at evaluation of relaxed growth restriction using fewer animals (Chapter 4). However, using more birds (replicates) in future studies is recommended to reach more powerful analysis using the response surface method. In the current study, important factors that affect biological and economic responses were identified, which were the amount of BW gain during the prepubertal growth phase (g_1) and pubertal growth phase inflection time (I_2). The next step is to determine any combination of growth model parameters that could result in the optimum values of multiple sets of responses. The optimum value of the response may either be a maximum value or a minimum value, depending upon the product or process in question. For example, if the response in an experiment is the yield of robust chicks, then the objective should be to find the settings of the factors affecting the yield so that the yield is maximized. On the other hand, if the response in an experiment is the number of defects (e.g., mortality rate, skeletal problems, etc.), then the goal would be to find the factor settings that minimize the number of defects. If there are multiple competing objectives, then optimization should be done to find a trade-off between the competing objectives. In fact, methodologies that are used to find optimum response are referred to as response surface method. This method is able to optimize a number of responses at the same time. For example, an experimenter may want to maximize strength (e.g., robust chick production), while keeping the number of defects (e.g. mortality) to a minimum.

Chapter 6 discussed the intergenerational effects of maternal growth trajectories in broiler breeders. The mechanism behind the effect of maternal environmental and nutritional conditions

would either be through altered egg composition (O'Sullivan et al., 1991; Ekmay et al., 2013, 2014) or epigenetic mechanisms (Ferguson-Smith, 2011). We investigated the effects of growth trajectories on egg weight and egg component weight. It would also be of great value to further evaluate the effects of BW targets on egg composition (e.g., fatty acids composition). Further studies can investigate epigenetic effect of growth patterns, which can be passed onto the offspring. Epigenetic mechanisms are defined as alterations in the gene expression profile of a cell that are not caused by changes in DNA sequence; DNA methylation is an example of an epigenetic mechanism (Otterdijk and Michels, 2016; Pang et al., 2017). Therefore, analysing the DNA methylation and histone modification patterns of offspring from feed restricted broiler breeders in various levels and ad libitum fed broiler breeders would provide valuable insight into underlying epigenetic mechanisms. We cooperated with colleagues in North Carolina State by providing breeder and offspring liver samples to explore differential expression of genes (through RNA sequencing) in the liver. The full results of their study will be available upon completion. Briefly, RNA sequencing results suggested that feed restriction on the broiler breeder generation affected the hepatic gene expression of both the broiler breeder generation experiencing the feed restriction and the subsequent progeny broiler generation. Additionally, DNMT3A and DNAMT3B, two of the main enzymes responsible for DNA methylation, both increased in a growth trajectory that was 20% above the standard BW curve (Figure 8.1 in Chapter 8). Their results pointed to possible epigenetic modifications being induced due to growth treatments. However, since epigenetic mechanisms such as DNA methylation and histone modification were not measured directly, further investigation into these mechanisms is needed for confirmation of epigenetic effects.

In Chapter 7 we described metabolome biomarkers for sexual development in broiler breeders. Financial budget permitting, one can investigate the effects of different growth trajectories on metabolome with a focus on activation of HPG axis during sexual maturity. The main question is what mechanisms are linking metabolic status and reproductive axis in broiler breeders? To find possible answers, investigating the signaling pathways integrated with activation of reproductive axis (e.g. gene expression of adipokines and their receptors in adipose tissue) in a cause-and-effect relationship would provide a better understanding of the physiological mechanisms driving the interaction among growth trajectory, feed allocation, sexual maturity, metabolic status, and reproductive axis. Such information will help the poultry industry optimize broiler breeder growth trajectories taking into account multiple biological and reproductive responses. As more evidence of convergence between hormones influencing both metabolic control and reproductive processes emerges, it is imperative to further study and describe these interactions to circumvent poor reproductive performance in broiler breeders. Such information can also be used in the field of human reproduction research. Research studies on female athletes have shown that lack of adequate body fat for reproduction leads to reproductive dysfunction such as delayed menarche (primary amenorrhoea) in girls, cessation of menses (secondary amenorrhoea) or sporadic menses (oligomenorrhoea) in adolescents and young women (Zanker, 2006). The author reported that a reduction in plasma leptin and adipokine concentrations below a critical threshold value in female athletes, for a significant period of time, disturbs the activity of the hypothalamic GnRH pulse generator. The same metabolites (adipokines) can be investigated in broiler breeder plasma and linked to the GnRH gene expression in the future studies. Therefore, further studies on integrated signaling pathways

with activation of the reproductive axis will help gynecologists and researchers in the field of human reproduction science to understand and overcome related reproductive issues.

In Chapter 8 we discussed the effects of photostimulation BW on the concentration of plasma CORT. Blood samples were taken from each bird between 1 to 3 h after the start of the photoperiod. However, diurnal variations in plasma CORT levels have been reported in several studies on laying hens (Johnson and van Tienhoven, 1981), broiler chickens (Lauber et al., 1987), broiler breeder pullets (Aranibar et al., 2020; Arrazola et al., 2019), and turkeys (Proudman, 1991). Although some studies indicated that feed restriction can act as a stress factor leading to increase blood CORT (Mench, 1991; Hocking et al., 1996; de Beer et al., 2008; van Krimpen and de Jong, 2014), recent studies have attributed the changes in plasma CORT level to differences in metabolic rate as well as differences in level of stress (Jimeno et al., 2020; Arrazola et al., 2019). A question that comes to mind is whether increased plasma CORT concentrations in feed restriction studies were due to psychological and behavioral stress, metabolic stress, or both. Some studies concluded that high levels of feed restriction require CORT regulation for glucose homeostasis during off-feed day in broiler breeders (Arrazola et al., 2019; Aranibar et al., 2020). Further research is required to elucidate the underlying mechanisms of the relationship among feed restriction, plasma CORT level, and metabolic rate in breeders. A possible approach to do this would be using different levels of feed restriction and various levels of dietary energy and protein using a multi-feeder PF system. It would also be prudent to investigate diurnal variations in plasma CORT level in breeders. An important consideration during blood collection is reducing blood sampling-associated stress, which otherwise could influence plasma CORT levels and interfere with treatment effects. To minimize interfering with the animal at the time of blood sampling, we recommend using automated blood sampling

technique through a vascular catheter. This technique has been validated for use in rats, with negligible impact on stress-associated hormone levels (Abelson et al., 2005; Siswanto et al., 2008), but it needs to be validated in poultry as well.

9.8 Conclusion

To decrease the gap between broiler breeders and their offspring target BW, and mitigate adverse effects of severe feed restriction, the current study was designed focusing on relaxed growth restriction during prepubertal growth phase and earlier pubertal growth phase. To our knowledge, this is the first systematic design of BW targets based on earlier pubertal growth phase in broiler breeders to find an optimum growth strategy. The strategy of earlier pubertal growth could reduce hunger in broiler breeders during both the rearing and laying phases. It also allows pullets to achieve a sufficient foundation and appropriate body fat level for sexual maturation, which can advance sexual maturation and ultimately, increase chick production. Increasing prepubertal and pubertal BW gains by more than 15% of the breeder-recommended target BW could trigger fat metabolism and yolk precursor synthesis, which consequently could advance sexual maturity. The current study suggests that raising the BW of broiler breeders in a precision feeding system increases reproductive performance of broiler breeders and growth performance of their offspring, which subsequently increases profitability for both breeder farmers and the supply chain as a whole. More specifically, the economic analysis of the current thesis predicted that increasing prepubertal BW gain by 10% and advancing the pubertal growth phase by 20% (scenario 10 in Table 9.3 and Figure 9.3), compared to the breeder-recommended target growth, could increase margin over feed and chick cost for hatching egg and broiler producers, and for the broiler chicken supply chain as a whole.

9.9 References

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9.10 Tables

Table 9. 1. Regression equations used in partial budget analysis to predict the number of settable egg production and ADFI (g/d) for each phase in Ross 708 broiler breeders.

Parameter of interest	Period (wk)	Predicting regression equation[1]
Rearing phase feed intake	3 to 6	$127.74 - (4.34 \times g_1) - (3.97 \times I_2) + (0.47 \times g_1 \times I_2)$
	7 to 10	$130.43 - (4.34 \times g_1) - (3.97 \times I_2) + (0.47 \times g_1 \times I_2)$
	11 to 14	$133.33 - (4.34 \times g_1) - (3.97 \times I_2) + (0.47 \times g_1 \times I_2)$
	15 to 18	$151.14 - (4.34 \times g_1) - (3.97 \times I_2) + (0.47 \times g_1 \times I_2)$
	19 to 22	$166.65 - (4.34 \times g_1) - (3.97 \times I_2) + (0.47 \times g_1 \times I_2)$
Laying phase feed intake	23 to 26	$69.19 + (61.63 \times g_1) + (1.39 \times I_2) - (2.66 \times g_1 \times I_2)$
	27 to 30	$115.66 + (61.63 \times g_1) + (1.39 \times I_2) - (2.66 \times g_1 \times I_2)$
	31 to 34	$127.75 + (61.63 \times g_1) + (1.39 \times I_2) - (2.66 \times g_1 \times I_2)$
	35 to 38	$106.41 + (61.63 \times g_1) + (1.39 \times I_2) - (2.66 \times g_1 \times I_2)$
	39 to 42	$106.21 + (61.63 \times g_1) + (1.39 \times I_2) - (2.66 \times g_1 \times I_2)$
Settable egg production	23 to 26	$-1.12 + (18.11 \times g_1) + (0.35 \times I_2) - (0.86 \times g_1 \times I_2)$
	27 to 30	$15.91 + (18.11 \times g_1) + (0.35 \times I_2) - (0.86 \times g_1 \times I_2)$
	31 to 34	$12.62 + (18.11 \times g_1) + (0.35 \times I_2) - (0.86 \times g_1 \times I_2)$
	35 to 38	$16.68 + (18.11 \times g_1) + (0.35 \times I_2) - (0.86 \times g_1 \times I_2)$
	39 to 42	$14.97 + (18.11 \times g_1) + (0.35 \times I_2) - (0.86 \times g_1 \times I_2)$

[1] Regression equations were derived based on the feed intake and settable egg production responses of Ross 708 broiler breeders raised under ten unique growth trajectories. A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. BW trajectories were designed with two levels of prepubertal BW gain (g_1) coefficient and 5 levels of pubertal growth phase inflection point (I_2) coefficient. g_1 was estimated from the breeder-recommended standard BW gain (Standard g_1) target, or 10% higher (High g_1). Second growth phase (pubertal) inflection point (I_2) was advanced such that I_2-0% = 22.29 wk, I_2-5% = 21.16 wk, I_2-10% = 20.05 wk, I_2-15% = 18.94 wk, I_2-20% = 17.82 wk. To use the regression equations, g_1 is 0 and 1 for the Standard g_1 and the

High g_1 treatments, respectively. I_2 is the pubertal phase inflection time (wk), which takes one of the following numbers: 17.82, 18.94, 20.05, 21.16, and 22.29.

Table 9. 2. Regression equations used in partial budget analysis to predict BW 35 d and cumulative feed intake (g) until 35 d of age in broilers originated from broiler breeders raised under various growth trajectories[1].

Parameter of interest	Predicting regression equation[1]
BW 35 d (g)	$1479.88 + (408.62 \times MW) + (17.29 \times MI) - (19.91 \times MW \times MI) + (993.71 \times Sex) + (750.44 \times MW \times Sex) - (43.23 \times MI \times Sex) - (35.77 \times MW \times MI \times Sex) + (51.88 \times MA)$
Cumulative feed intake (g)	$2094.99 + (539.80 \times MW) + (26.12 \times MI) - (27.74 \times MW \times MI) + (604.29 \times Sex) + (2203.05 \times MW \times Sex) - (25.18 \times MI \times Sex) - (105.11 \times MW \times MI \times Sex) - (88.97 \times MA)$

[1] Regression equations were derived based on the cumulative feed intake until 35 d and BW 35 d responses of Ross 708 broiler chickens. The broilers originated from two maternal ages (MA= 35 and 42 wk) of broiler breeders that raised under ten unique maternal growth trajectories. A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. Maternal BW trajectories were designed with two levels of maternal prepubertal BW gain (MW) coefficient and 5 levels of maternal pubertal growth phase inflection point (MI) coefficient. MW was estimated from the breeder-recommended standard maternal BW gain (Standard MW) target, or 10% higher (High MW). Second maternal growth phase (pubertal) inflection point (MI) was advanced such that MI-0% = 22.29 wk, MI-5% = 21.16 wk, MI-10% = 20.05 wk, MI-15% = 18.94 wk, MI-20% = 17.82 wk. To use the regression equations, MW is 0 and 1 for the Standard MW and the High MW treatments, respectively. MI is the pubertal phase inflection time (wk), which takes one of the following numbers: 17.82, 18.94, 20.05, 21.16, and 22.29. MA is 0 and 1 for the maternal age of 35 and 42 wk.

Table 9. 3. Target BW in a range of growth scenarios[1] in broiler breeders from 0 to 42 wk of age.

	Growth Scenario									
Age	1	2	3	4	5	6	7	8	9	10
wk	kg									
0	0.100	0.100	0.100	0.100	0.100	0.110	0.110	0.110	0.110	0.110
1	0.150	0.150	0.150	0.150	0.150	0.165	0.165	0.165	0.165	0.165
2	0.212	0.212	0.212	0.212	0.212	0.233	0.233	0.233	0.233	0.233
3	0.285	0.285	0.285	0.285	0.285	0.314	0.314	0.314	0.314	0.314
4	0.370	0.370	0.370	0.370	0.370	0.407	0.407	0.407	0.407	0.407
5	0.462	0.462	0.462	0.462	0.462	0.508	0.508	0.508	0.508	0.508
6	0.560	0.560	0.560	0.560	0.560	0.616	0.616	0.616	0.616	0.616
7	0.661	0.661	0.661	0.661	0.661	0.727	0.727	0.727	0.727	0.727
8	0.763	0.763	0.763	0.763	0.764	0.839	0.839	0.839	0.839	0.840
9	0.863	0.863	0.863	0.864	0.867	0.950	0.950	0.950	0.950	0.953
10	0.961	0.961	0.961	0.963	0.971	1.057	1.057	1.057	1.059	1.067
11	1.053	1.054	1.056	1.062	1.080	1.158	1.159	1.161	1.168	1.186
12	1.141	1.142	1.148	1.165	1.199	1.255	1.256	1.262	1.279	1.313
13	1.223	1.228	1.243	1.275	1.332	1.345	1.350	1.365	1.398	1.454
14	1.302	1.316	1.346	1.399	1.480	1.431	1.445	1.475	1.529	1.610
15	1.381	1.409	1.460	1.538	1.643	1.518	1.546	1.597	1.675	1.780
16	1.467	1.515	1.591	1.693	1.817	1.609	1.658	1.733	1.835	1.960
17	1.564	1.637	1.736	1.859	1.996	1.712	1.785	1.884	2.007	2.144
18	1.677	1.774	1.895	2.031	2.175	1.830	1.927	2.048	2.184	2.328
19	1.807	1.925	2.061	2.204	2.347	1.964	2.083	2.218	2.361	2.504
20	1.951	2.085	2.228	2.371	2.508	2.112	2.246	2.389	2.532	2.670
21	2.105	2.247	2.391	2.529	2.657	2.270	2.412	2.556	2.694	2.821
22	2.263	2.407	2.546	2.674	2.790	2.430	2.574	2.713	2.842	2.958
23	2.418	2.558	2.688	2.805	2.908	2.589	2.729	2.859	2.976	3.078
24	2.567	2.699	2.817	2.921	3.011	2.740	2.871	2.990	3.094	3.184
25	2.706	2.826	2.932	3.023	3.100	2.881	3.001	3.106	3.197	3.275
26	2.832	2.939	3.032	3.110	3.176	3.008	3.115	3.208	3.287	3.353
27	2.944	3.038	3.118	3.185	3.241	3.122	3.216	3.296	3.363	3.419
28	3.043	3.124	3.192	3.249	3.296	3.222	3.303	3.372	3.429	3.475
29	3.128	3.198	3.256	3.303	3.342	3.309	3.378	3.436	3.484	3.523
30	3.201	3.260	3.309	3.349	3.381	3.383	3.442	3.490	3.530	3.562
31	3.263	3.313	3.354	3.387	3.413	3.446	3.495	3.536	3.569	3.596
32	3.316	3.357	3.391	3.418	3.440	3.499	3.541	3.574	3.602	3.624
33	3.360	3.395	3.423	3.445	3.463	3.544	3.579	3.606	3.629	3.647
34	3.397	3.426	3.449	3.467	3.482	3.582	3.610	3.633	3.652	3.666
35	3.428	3.452	3.471	3.486	3.498	3.613	3.637	3.656	3.671	3.683
36	3.455	3.474	3.489	3.502	3.512	3.640	3.659	3.675	3.687	3.697
37	3.477	3.492	3.505	3.515	3.523	3.662	3.678	3.691	3.701	3.709
38	3.496	3.508	3.519	3.527	3.534	3.682	3.694	3.705	3.713	3.720
39	3.512	3.523	3.531	3.538	3.543	3.698	3.709	3.717	3.724	3.729
40	3.527	3.535	3.542	3.548	3.552	3.713	3.722	3.729	3.734	3.738
41	3.540	3.547	3.553	3.557	3.561	3.727	3.734	3.739	3.744	3.747

42	3.553	3.558	3.563	3.567	3.569	3.739	3.745	3.750	3.753	3.756

[1]A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. BW trajectories were designed with two levels of prepubertal BW gain (g_1) coefficient and 5 levels of pubertal growth phase inflection point (I_2) coefficient. g_1 was estimated from the breeder-recommended standard BW gain (Standard g_1) target, or 10% higher (High g_1). Second growth phase (pubertal) inflection point (I_2) was advanced such that I_2-0% = 22.29 wk, I_2-5% = 21.16 wk, I_2-10% = 20.05 wk, I_2-15% = 18.94 wk, I_2-20% = 17.82 wk. Scenario 1 = Standard g_1, I_2-0%; Scenario 2 = Standard g_1, I_2-5%; Scenario 3 = Standard g_1, I_2-10%; Scenario 4 = Standard g_1, I_2-15%; Scenario 5 = Standard g_1, I_2-20%; Scenario 6 = High g_1, I_2-0%; Scenario 7 = High g_1, I_2-5%; Scenario 8 = High g_1, I_2-10%; Scenario 9 = High g_1, I_2-15%; Scenario 10 = High g_1, I_2-20%.

9.11 Figures

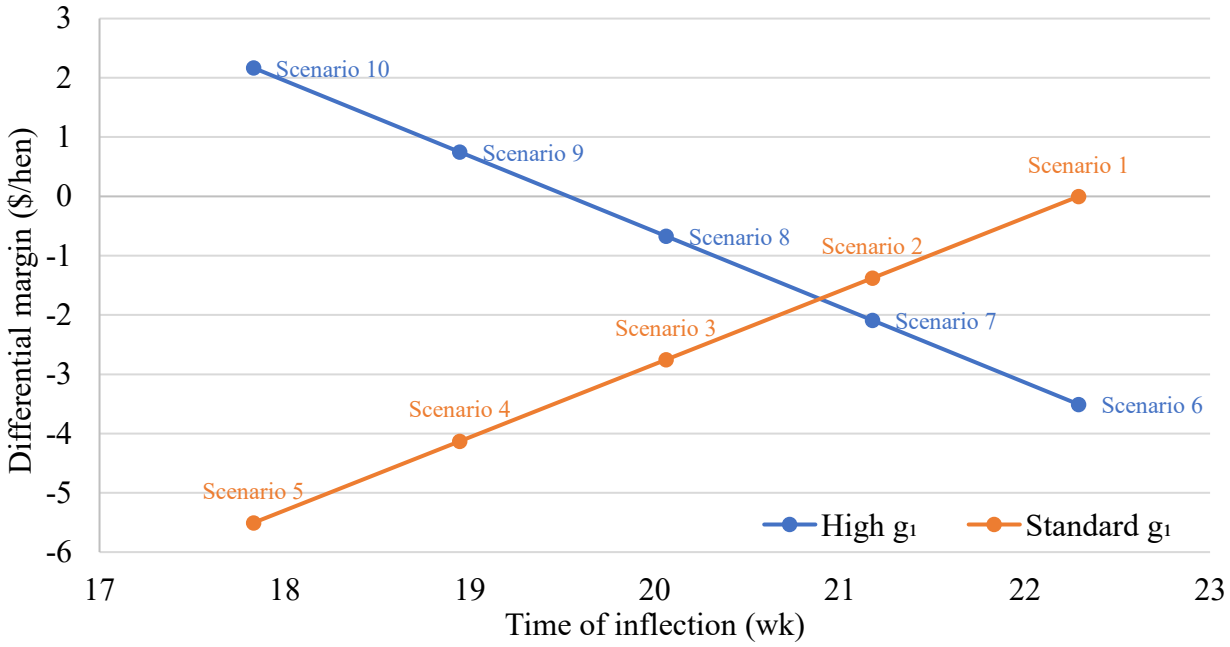

Figure 9. 1. Differential margin over feed and pullet cost in broiler breeder sector between each growth scenario and the Ross 708 broiler breeder-recommended growth trajectory (scenario 1). The margin has been calculated based on the number of saleable chick production (income) over the pullet cost and feed cost from 3 to 42 wk of age. A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. BW trajectories were designed with two levels of prepubertal BW gain (g_1) coefficient and 5 levels of pubertal growth phase inflection point (I_2) coefficient. g_1 was estimated from the breeder-recommended standard BW gain (Standard g_1) target, or 10% higher (High g_1). Second growth phase (pubertal) inflection point (I_2) was advanced such that I_2-0% = 22.29 wk, I_2-5% = 21.16 wk, I_2-10% = 20.05 wk, I_2-15% = 18.94 wk, I_2-20% = 17.82 wk. Scenario 1 = Standard g_1, I_2-0%; Scenario 2 = Standard g_1, I_2-5%; Scenario 3 = Standard g_1, I_2-10%; Scenario 4 = Standard g_1, I_2-15%; Scenario 5 = Standard g_1, I_2-20%; Scenario 6 = High g_1, I_2-0%; Scenario 7 = High g_1, I_2-5%; Scenario 8 = High g_1, I_2-10%; Scenario 9 = High g_1, I_2-15%; Scenario 10 = High g_1, I_2-20%.

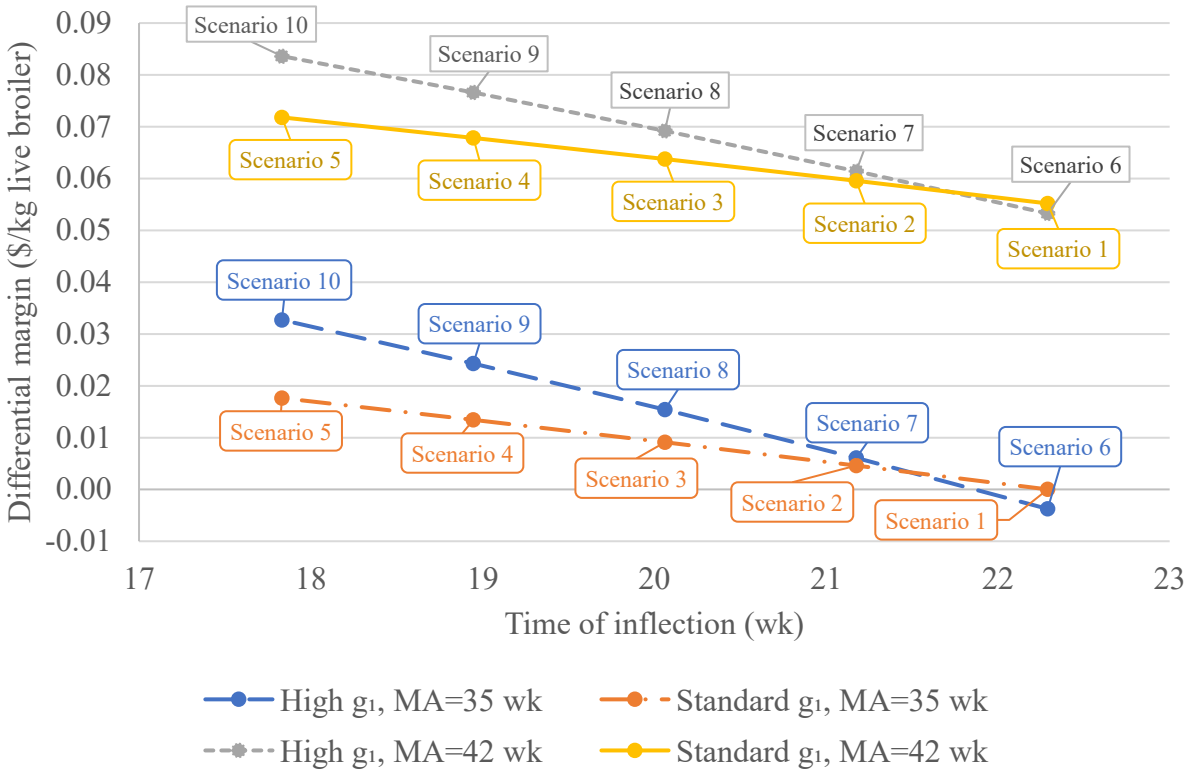

Figure 9. 2. Differential margin over feed and chick cost in supply chain (broiler) sector between each maternal growth scenario and the breeder-recommended maternal growth trajectory (scenario 1). The margin has been calculated based on the broilers live BW at 35 d (income) over the chick cost and feed cost from 0 to 35 d of age. A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. Maternal BW trajectories were designed with two levels of maternal prepubertal BW gain (MW) coefficient and 5 levels of maternal pubertal growth phase inflection point (MI) coefficient. MW was estimated from the breeder-recommended standard maternal BW gain (Standard MW) target, or 10% higher (High MW). Second maternal growth phase (pubertal) inflection point (MI) was advanced such that MI-0% = 22.29 wk, MI-5% = 21.16 wk, MI-10% = 20.05 wk, MI-15% = 18.94 wk, MI-20% = 17.82 wk. Chicks were from two maternal ages (MA= 35 and 42 wk). Scenario 1 = Standard MW, MI-0%; Scenario 2 = Standard MW, MI-5%; Scenario 3 = Standard MW, MI-10%; Scenario 4 = Standard MW, MI-15%; Scenario 5 = Standard MW, MI-20%; Scenario 6 = High MW, MI-0%; Scenario 7 = High MW, MI-5%; Scenario 8 = High MW, MI-10%; Scenario 9 = High MW, MI-15%; Scenario 10 = High MW, MI-20%.

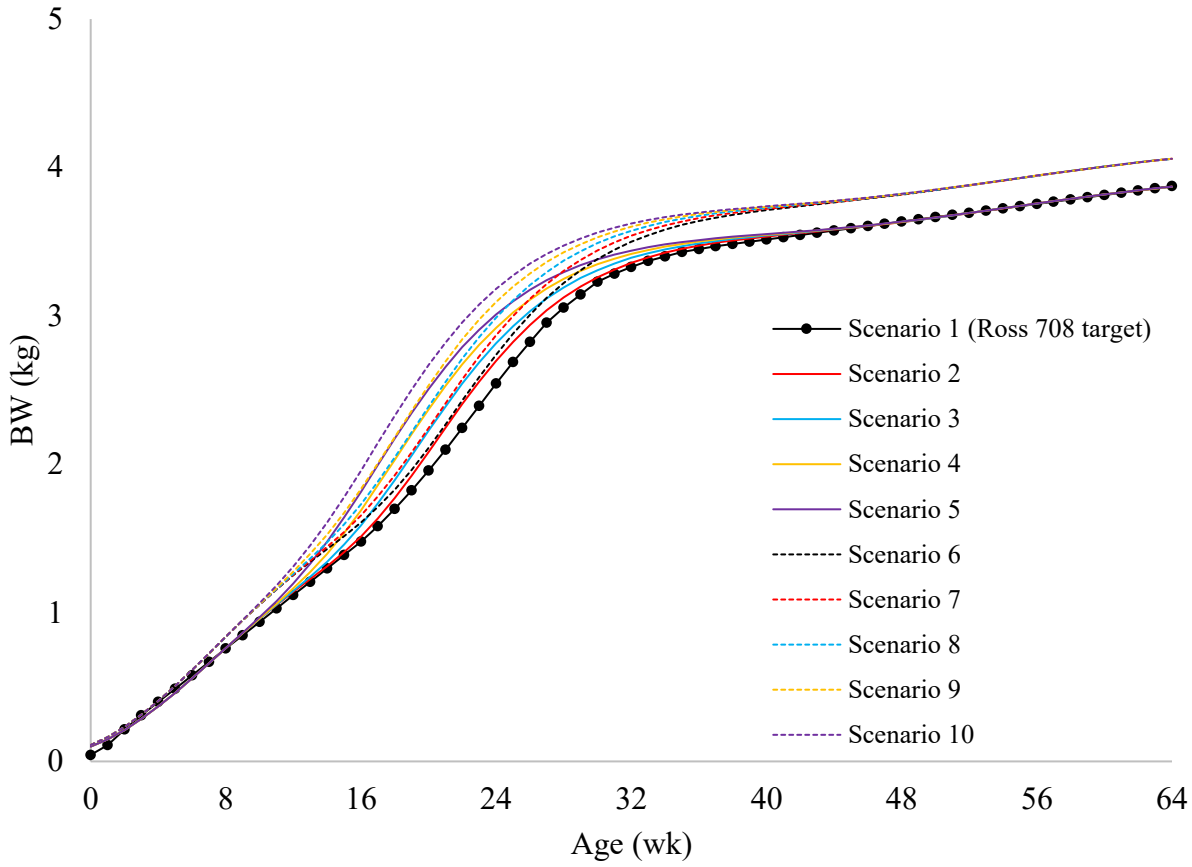

Figure 9. 3. Growth trajectories designed using estimated coefficients of a 3 phase Gompertz model. General model form was $BW_t = \sum_{i=1}^{i=3} g_i exp^{-exp^{-b_i(t-I_i)}}$ where BW_t was BW (kg) at time t (wk); g_i was the total amount of gain (kg) in phase i ; b_i was the growth rate coefficient; t was age (wk); I_i was the inflection point (wk), or the age at which growth for phase i reached its maximum rate. g_1 coefficient (g_1) was the prepubertal phase gain coefficient estimated by fitting the model to the standard Ross 708 recommended BW gain target (Standard g_1) or 10% higher (High g_1). Pubertal phase inflection point coefficient (I_2) was advanced by 5, 10, 15, and 20% creating inflection points at 21.16, 20.05, 18.94, and 17.82 wk of age, respectively. Scenario 1 (Ross 708 target) = Standard g_1, I_2-0%; Scenario 2 = Standard g_1, I_2-5%; Scenario 3 = Standard g_1, I_2-10%; Scenario 4 = Standard g_1, I_2-15%; Scenario 5 = Standard g_1, I_2-20%; Scenario 6 = High g_1, I_2-0%; Scenario 7 = High g_1, I_2-5%; Scenario 8 = High g_1, I_2-10%; Scenario 9 = High g_1, I_2-15%; Scenario 10 = High g_1, I_2-20%.

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Appendix A

Partial budgeting allows us to understand how a decision will affect the profitability of an enterprise. Partial budgeting is a systematic approach that can assist the manager in making informed decisions. However, this budgeting process can only estimate possible financial impacts, not assure them. Repeating the analysis using different assumptions about key variables will give some idea about the degree of risk (the probability that actual outcomes will differ from expected ones) involved in making the proposed change.

Partial Budget Analysis for Hatching Egg Producer Sector Using Different Growth Trajectory Scenarios Compared with the Ross 708 Breeder-Recommended Scenario

In this section, economic projections of switching broiler breeder target growth from the breeder-recommended target growth (scenario 1) to 9 alternative growth scenarios (scenarios 2 to 10) are investigated. The explanation on creating the growth scenarios was provided in Chapter 9.

The partial budget model was created in an Excel spreadsheet (Tables AA.1 and AA.2). The following steps were taken to run economic analysis:

1. Settable eggs were defined as the eggs heavier than 52 g. The number of saleable chicks for each growth scenario was calculated by multiplying the settable egg numbers of each scenario (estimated from regression equations in Table 9.1) by 91% (assumed saleable chicks as a percentage of settable eggs).
2. Analysis of covariance was conducted on the number of saleable chicks and ADFI using the MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC), with prepubertal phase gain (g_1) and time period as discrete sources of variation, and pubertal

growth inflection time (I_2) as a continuous predictor variable (Chapter 4). This analysis created coefficients for the response predicting equations described in Table 9.1 for each 4-wk period.

3. The numbers of settable eggs during the laying phase and ADFI (g/d) of broiler breeders during the rearing and the laying phase was estimated for each growth scenario using regression equations created based on the experimental data (Table 9.1). These predictions are shown in Table AA.1.
4. Based on the estimated responses shown in Table AA.1 and the assumptions provided in Table AA.2, the partial budget model was developed for the broiler breeder sector (Table AA.3).
5. Margin over feed and pullet cost ($/hen) for each growth scenario was calculated by the following formula:

$$Margin = Revenue_1 - Cost_1 - Cost_2$$

Where Margin was margin over feed and pullet cost ($/hen); $Revenue_1$ was revenue from saleable chick ($/hen); $Cost_1$ was feed cost during the rearing and laying phases ($/hen); and $Cost_2$ was pullet cost ($/hen).

6. Differential margin over feed and pullet cost ($/hen) for each growth scenario was calculated by subtracting the margin ($/hen) of scenario 1 (breeder-recommended growth trajectory) from the margin ($/hen) of each alternative scenario.
7. Pullet cost per saleable chick ($/chick) for each growth scenario was calculated by dividing pullet cost by the estimated numbers of saleable chicks for each growth scenario. This rewards higher chick production by dividing the pullet placement costs over the

number of saleable chicks, which was expected to differ among scenarios. The following formula was used:

$$\text{Margin over feed and pullet cost per each saleable chick (\$/chick)} = \frac{\text{Margin (\$/hen)}}{Saleable\ chicks\ (/hen)} - pullet\ cost\ per\ chick\ (\$/pullet)$$

8. Differential margin ($/chick) was calculated for each growth scenarios by subtracting the margin over feed and pullet cost ($/chick) of the scenario 1 (breeder-recommended growth trajectory) from the margin over feed and pullet cost ($/chick) of each alternative scenario.
9. Chick cost ($/chick) was calculated for each growth scenario by dividing the feed cost during rearing and laying phases of that scenario by the relevant number of saleable chicks.
10. Differential chick cost ($/chick) was calculated for each growth scenario by subtracting the chick cost of scenario 1 from the chick cost ($/chick) of each alternative scenario. Differential chick cost values were used in the partial budget model for the offspring (broiler) sector in appendix B.

Table AA. 1. Estimated production responses of broiler breeders to the growth scenarios[1] using the regression equations[2] developed from experimental data.

Growth scenario	Prepubertal gain (g_1)	Pubertal inflection time (I_2)	Saleable chick (number)	ADFI (g/d)	Feed intake (kg/hen/period)	ADFI (g/d)	Feed intake (kg/hen/period)
		wk	up to 42 wk	3 to 21wk		22 to 42wk	
1	Standard	22.29	89.3	53.3	7.09	136.0	19.04
2	Standard	21.17	87.5	57.8	7.68	134.4	18.82
3	Standard	20.06	85.7	62.2	8.27	132.9	18.60
4	Standard	18.94	83.9	66.6	8.86	131.3	18.39
5	Standard	17.83	82.2	71.0	9.45	129.8	18.17
6	High	22.29	84.9	59.5	7.91	138.2	19.35
7	High	21.17	87.5	63.4	8.43	139.6	19.55
8	High	20.06	90.0	67.3	8.95	141.1	19.75
9	High	18.94	92.6	71.2	9.47	142.5	19.95
10	High	17.83	95.2	75.1	9.99	143.9	20.15

[1]A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. BW trajectories were designed with two levels of prepubertal BW gain (g_1) coefficient and 5 levels of pubertal growth phase inflection point (I_2) coefficient. g_1 was estimated from the breeder-recommended standard BW gain (Standard g_1) target, or 10% higher (High g_1). Second growth phase (pubertal) inflection point (I_2) was advanced such that I_2-0% = 22.29 wk, I_2-5% = 21.16 wk, I_2-10% = 20.05 wk, I_2-15% = 18.94 wk, I_2-20% = 17.82 wk. Scenario 1 = Standard g_1, I_2-0%; Scenario 2 = Standard g_1, I_2-5%; Scenario 3 = Standard g_1, I_2-10%; Scenario 4 = Standard g_1, I_2-15%; Scenario 5 = Standard g_1, I_2-20%; Scenario 6 = High g_1, I_2-0%; Scenario 7 = High g_1, I_2-5%; Scenario 8 = High g_1, I_2-10%; Scenario 9 = High g_1, I_2-15%; Scenario 10 = High g_1, I_2-20%.

[2]Analysis of covariance was conducted on the number of saleable chick and ADFI using the MIXED procedure of SAS, with prepubertal phase gain (g_1) and time period as discrete sources of variation, and pubertal growth inflection time (I_2) as a continuous predictor variable to estimate coefficients of response predicting regression equations.

Table AA. 2. Price assumptions used in the broiler breeder partial budget analysis

Item	Price
Broiler breeder starter feed ($/kg)	0.57
Broiler breeder pullet developer feed ($/kg)	0.45
Broiler breeder peak lay feed ($/kg)	0.50
Saleable chick ($/each)	0.68
Pullet cost ($/each)	10.00

Table AA. 3. Partial budget analysis for hatching egg producer sector using different growth trajectory scenarios[1] compared with the Ross 708 breeder-recommended scenario (Scenario 1).

	Revenue	Cost			Margin over feed and pullet cost				Cost	
Growth scenario	Saleable chicks (up to 42 wk)	Feed (3 to 21wk)	Feed (22 to 42wk)	Pullet cost/saleable chick	Margin	Differential margin [2]	Margin	Differential margin [2]	Chick cost	Differential chick cost [2]
	$/hen			$/chick	($/hen)		($/chick)			
1	61.11	3.22	9.44	0.1319	36.67	0.00	0.2788	0.0000	0.1418	0.0000
2	59.90	3.49	9.33	0.1346	35.30	-1.38	0.2687	-0.0101	0.1465	0.0047
3	58.68	3.76	9.22	0.1374	33.92	-2.75	0.2583	-0.0206	0.1514	0.0096
4	57.46	4.02	9.12	0.1403	32.54	-4.13	0.2473	-0.0315	0.1565	0.0147
5	56.25	4.29	9.01	0.1434	31.17	-5.51	0.2359	-0.0429	0.1619	0.0201
6	58.13	3.59	9.59	0.1387	33.16	-3.51	0.2518	-0.0270	0.1553	0.0135
7	59.88	3.83	9.69	0.1347	34.58	-2.09	0.2606	-0.0182	0.1546	0.0128
8	61.64	4.07	9.79	0.1308	36.00	-0.67	0.2690	-0.0098	0.1539	0.0121
9	63.39	4.30	9.89	0.1272	37.42	0.75	0.2769	-0.0019	0.1532	0.0114
10	65.14	4.54	9.99	0.1238	38.84	2.17	0.2843	0.0055	0.1526	0.0108

[1]A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. BW trajectories were designed with two levels of prepubertal BW gain (g_1) coefficient and 5 levels of pubertal growth phase inflection point (I_2) coefficient. g_1 was estimated from the breeder-recommended standard BW gain (Standard g_1) target, or 10% higher (High g_1). Second growth phase (pubertal) inflection point (I_2) was advanced such that I_2-0% = 22.29 wk, I_2-5% = 21.16 wk, I_2-10% = 20.05 wk, I_2-15% = 18.94 wk, I_2-20% = 17.82 wk. Scenario 1 = Standard g_1, I_2-0%; Scenario 2 = Standard g_1, I_2-5%; Scenario 3 = Standard g_1, I_2-10%; Scenario 4 = Standard g_1, I_2-15%; Scenario 5 = Standard g_1, I_2-20%; Scenario 6 = High g_1, I_2-0%; Scenario 7 = High g_1, I_2-5%; Scenario 8 = High g_1, I_2-10%; Scenario 9 = High g_1, I_2-15%; Scenario 10 = High g_1, I_2-20%.

[2]Differential margins and differential cost for each growth scenario were calculated by subtracting margin and cost of scenario 1 from those of each growth scenario.

Appendix B

Partial Budget Analysis for Broiler Sector Using Different Maternal Growth Trajectory Scenarios Compared with the Ross 708 Breeder-Recommended Scenario

In this section, economic projections of switching maternal target growth from the breeder-recommended target growth (scenario 1) to 9 alternative maternal growth scenarios (scenario 2 to 10) are investigated for the broiler sector.

The partial budget model was created in an Excel spreadsheet (Tables AB.1 and AB.2). The following steps were taken to run economic analysis:

1. Analysis of covariance was conducted on all dependent variables using the MIXED procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC), with broiler sex, maternal age and maternal prepubertal gain (MW) as sources of variation, maternal pubertal inflection (MI) as a continuous predictor variable, and dam as a random subject (Chapter 6). This analysis created coefficients for the response predicting equations described in Table 9.2.
2. Broiler 35 d BW and cumulative feed intake were estimated for each growth scenario using the regression equations created based on the experimental data (Table 9.2). The results of these predictions are shown in Table AB.1.
3. Based on the estimated responses shown in Table AB.1 and the assumptions provided in Table AB.2, the partial budget model was developed (Table AB.3). Note that the sex ratio for male to female broilers was assumed to be 50:50.

4. Revenue of selling broiler chicken at 35 d and feed cost ($/chicken) were calculated by multiplying the estimated responses by chicken and feed prices, respectively (Table AB.3), using the formula:

$$Revenue\ from\ broiler\ (\$\ /\ chicken) = 35d\ BW\ \times broiler\ price$$

$$Feed\ cost\ (\$\ /\ chicken) = Feed\ intake\ \times broiler\ feed\ cost$$

5. Differential chick cost ($/chick) for each growth scenario was calculated in partial budget of the breeder sector (Table AA.3) and used in partial budget of the broiler sector. Inclusion of differential chick cost in calculating margin over feed and chick cost accounts for the differences in chick cost of each growth scenario and the growth scenario 1. Differential chick cost is important for supply chain level economic analysis. Thus, we used the industry chick price plus the differential chick price (calculated from the breeder sector) as well as feed cost to define the cost. Therefore, margin for the broiler sector has been defined as margin over feed and chick cost.
6. Margin over feed and chick ($/chicken) for each growth scenario was calculated by subtracting the feed and differential chick cost of alternative scenario and the chick cost from the revenue of selling broiler chicken.
7. Margin over feed and chick ($/kg live chicken) for each growth scenario was calculated by dividing the margin ($/chicken) by the 35 d BW of that scenario.
8. The margin over feed and chick cost estimated for maternal growth scenario 1 (breeder-recommended scenario) from 35-wk old hens was used as a reference to compare the margin of other maternal growth scenarios (from 35- and 42-wk old hens). In other words, differential margin over feed and chick cost ($/kg live) was calculated for each growth scenario using the following formula:

$$Differential\ Margin = Margin_1 - Margin_2$$

Where Differential Margin ($/kg live chicken) over feed and chick cost was the difference between margin of each maternal growth scenario and the maternal growth scenario 1 (breeder-recommended scenario from 35-wk old hens). $Margin_1$ was margin over feed, chick, and differential chick cost ($/kg live chicken) for each maternal growth scenari. $Margin_2$ was margin over feed, chick, and differential chick cost ($/kg live chicken) for the maternal growth scenario 1.

Table AB. 1. Estimated production responses of 35 d old broilers to the maternal growth scenarios[1] using the regression equations[2] developed from offspring experimental data.

Growth scenario	Maternal prepubertal gain (g_1)	Maternal pubertal inflection time (I_2)	Maternal age	35d BW	Feed intake
		wk		g	
1	Standard	22.29	35	1,880	2,699
2	Standard	21.17	35	1,885	2,684
3	Standard	20.06	35	1,890	2,669
4	Standard	18.94	35	1,895	2,654
5	Standard	17.83	35	1,900	2,638
6	High	22.29	35	1,822	2,550
7	High	21.17	35	1,869	2,625
8	High	20.06	35	1,916	2,699
9	High	18.94	35	1,963	2,774
10	High	17.83	35	2,010	2,848
1	Standard	22.29	42	1,932	2,610
2	Standard	21.17	42	1,937	2,595
3	Standard	20.06	42	1,942	2,580
4	Standard	18.94	42	1,947	2,565
5	Standard	17.83	42	1,951	2,549
6	High	22.29	42	1,874	2,461
7	High	21.17	42	1,921	2,536
8	High	20.06	42	1,968	2,610
9	High	18.94	42	2,014	2,685
10	High	17.83	42	2,061	2,759

[1]A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. Maternal BW trajectories were designed with two levels of maternal prepubertal BW gain (MW) coefficient and 5 levels of maternal pubertal growth phase inflection point (MI) coefficient. MW was estimated from the breeder-recommended standard maternal BW gain (Standard MW) target, or 10% higher (High MW). Second maternal growth phase (pubertal) inflection point (MI) was advanced such that MI-0% = 22.29 wk, MI-5% = 21.16 wk, MI-10% = 20.05 wk, MI-15% = 18.94 wk, MI-20% = 17.82 wk. Chicks were from two maternal ages (MA= 35 and 42 wk). Scenario 1 = Standard MW, MI-0%; Scenario 2 = Standard MW, MI-5%; Scenario 3 = Standard MW, MI-10%; Scenario 4 = Standard MW, MI-15%; Scenario 5 = Standard MW, MI-20%; Scenario 6 = High MW, MI-0%; Scenario 7 = High MW, MI-5%; Scenario 8 = High MW, MI-10%; Scenario 9 = High MW, MI-15%; Scenario 10 = High MW, MI-20%.

[2]Analysis of covariance was conducted on all dependent variables using the MIXED procedure of SAS, with broiler sex, maternal age and maternal prepubertal gain (MW) as sources of variation, maternal pubertal inflection (MI) as a continuous predictor variable, and dam as a random subject (Chapter 6). We created coefficients for the response predicting equations described in Table 9.2 from experimental data.

Table AB. 2. Price assumptions used in developing the broiler sector partial budget, assuming a male/female ratio of 50:50.

Item	Price
Day-old broiler chick ($/each)	0.844
Broiler feed (adjusted for starter, grower, and finisher phases ($/kg)	0.510
Broiler price ($/kg live)	1.895

Table AB. 3. Partial budget analysis for broiler sector using different maternal growth trajectory scenarios compared with the Ross 708 breeder-recommended scenario[1].

	Revenue	—— Cost ——		Margin over feed, chick, and differential chick cost		
Growth scenario	Broiler	Feed	Differential chick cost	Margin	Margin	Differential Margin
	——————	$/chicken	————————		— $/kg live chicken –	
1	3.56	1.38	0.0000	1.3428	0.7142	0.0000
2	3.57	1.37	0.0047	1.3550	0.7188	0.0046
3	3.58	1.36	0.0096	1.3669	0.7232	0.0091
4	3.59	1.35	0.0147	1.3786	0.7276	0.0134
5	3.60	1.35	0.0201	1.3901	0.7318	0.0176
6	3.45	1.30	0.0135	1.2942	0.7104	-0.0038
7	3.54	1.34	0.0128	1.3459	0.7202	0.0061
8	3.63	1.38	0.0121	1.3976	0.7296	0.0154
9	3.72	1.41	0.0114	1.4492	0.7384	0.0243
10	3.81	1.45	0.0108	1.5009	0.7469	0.0327
1	3.66	1.33	0.0000	1.4865	0.7693	0.0552
2	3.67	1.32	0.0047	1.4986	0.7737	0.0595
3	3.68	1.32	0.0096	1.5106	0.7779	0.0637
4	3.69	1.31	0.0147	1.5223	0.7820	0.0678
5	3.70	1.30	0.0201	1.5338	0.7860	0.0718
6	3.55	1.26	0.0135	1.4379	0.7674	0.0533
7	3.64	1.29	0.0128	1.4896	0.7756	0.0614
8	3.73	1.33	0.0121	1.5413	0.7833	0.0692
9	3.82	1.37	0.0114	1.5929	0.7907	0.0766
10	3.91	1.41	0.0108	1.6445	0.7978	0.0836

[1]A 3-phase Gompertz growth model was fitted to the Ross 708 female broiler breeder recommended target BW to estimate the model coefficients. Maternal BW trajectories were designed with two levels of maternal prepubertal BW gain (MW) coefficient and 5 levels of maternal pubertal growth phase inflection point (MI) coefficient. MW was estimated from the breeder-recommended standard maternal BW gain (Standard MW) target, or 10% higher (High MW). Second maternal growth phase (pubertal) inflection point (MI) was advanced such that MI-0% = 22.29 wk, MI-5% = 21.16 wk, MI-10% = 20.05 wk, MI-15% = 18.94 wk, MI-20% = 17.82 wk. Chicks were from two maternal ages (MA= 35 and 42 wk). Scenario 1 = Standard MW, MI-0%; Scenario 2 = Standard MW, MI-5%; Scenario 3 = Standard MW, MI-10%; Scenario 4 = Standard MW, MI-15%; Scenario 5 = Standard MW, MI-20%; Scenario 6 = High MW, MI-0%; Scenario 7 = High MW, MI-5%; Scenario 8 = High MW, MI-10%; Scenario 9 = High MW, MI-15%; Scenario 10 = High MW, MI-20%.

[2]Differential margins for each growth scenario were calculated by subtracting margin of maternal growth scenario 1 from those of each growth scenario.