Effect of Metabolizable Energy Intake on Metabolism and Reproduction of Broiler Breeders Fed by a Precision Feeding System

By

SHEILA HADINIA

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

DOCTOR OF PHILOSOPHY

In

ANIMAL SCIENCE

Department of Agricultural, Food and Nutritional Science

University of Alberta

Abstract

The objectives of the first study were to investigate metabolizable energy (**ME**) partitioning and energetic efficiency in Ross 308 broiler breeders. An empirical ME model was derived to describe ME used for total heat production (**HP**), growth (**ADG**), and hen-day egg production (**HDEP**). From 10 to 23 wk of age, pullets were assigned to 2 treatments: precision feeding (**PF**) or conventional skip-a-day feeding (**CON**). From 23 to 34 wk of age, the CON birds were fed on conventional daily restricted feeding; the PF system allowed birds to enter stations voluntarily at any time if their BW was less than the target BW. Energetic efficiency was evaluated using residual feed intake (**RFI**), defined as the difference between observed and predicted ME intake (**MEI**). From 10 to 23 wk of age, for CON and PF treatments, respectively, MEI was 194 and 174 kcal/d ($P < 0.001$); ADG was 15.3 and 15.4 g/d ($P = 0.94$); HP was 129 and 111 kcal/kg$^{0.68}$ ($P < 0.001$); FCR was 4.888 and 4.057 ($P < 0.001$). The CON pullets had similar ADG, but higher MEI and higher cumulative FCR relative to PF pullets. The PF pullets lost less energy as heat, likely because they were fed continuously, reducing the need to store and mobilize nutrients compared to CON pullets. Thus, increased feeding frequency increased PF pullet efficiency. From 23 to 34 wk of age, the energy partitioning model ($P < 0.05$) predicted $MEI = A \times BW^{0.67} + 1.75 \times ADG + 0.75 \times EM + \varepsilon$. The coefficient A was a vector of age-specific HP coefficient (155 kcal/kg$^{0.67}$/d); the energy requirement for growth and egg mass (**EM**) was 1.75 and 0.75 kcal/g respectively. Overall for CON and PF hens respectively, MEI was 366 and 354 kcal/d ($P = 0.006$); RFI was -5.9 and 6.7 kcal/d ($P = 0.01$); HP was 86.4 and 88.7%

of total MEI ($P < 0.001$); HDEP was 65.5 and 55.2% ($P < 0.001$). Although the CON hens had higher MEI, they lost less energy as HP, had more nutrients available for egg production and were more energetically efficient than the PF hens. Production-related feed increases for individual PF hens occurred only after they laid an egg, whereas feed allocation increases for the CON hens resulted in increasing MEI for all CON hens at the same time. Therefore, we hypothesized that current BW recommendations are too low for PF hens, and increasing MEI of the PF hens before they reach onset of lay is likely needed to stimulate onset of production. To assess the effect of MEI on rate of sexual maturation, a second study was conducted. Ross 308 broiler breeder pullets were assigned to two treatments from 22 to 26 wk of age and fed using a PF system: 1) **Low MEI** (2,807 kcal/kg diet, feed restricted), and 2) **High MEI** (3,109 kcal/kg diet, not restricted). Daily cloacal palpation was used to detect sexual maturity via the presence of a hard-shelled egg in the shell gland. Expression of gonadotropin releasing hormone-I (**GnRH**) and gonadotropin inhibitory hormone (**GnIH**) genes in hypothalamus, and GnRH receptor (**GnRH-RI**) and GnIH receptor (**GnIH-R**) genes in the anterior pituitary gland of each pullet was evaluated from 22 to 26 wk of age using quantitative real time-PCR. Blood samples were taken weekly and luteinizing hormone (**LH**), follicle stimulating-hormone (**FSH**) and 17-beta-estradiol (**E2**) determined using commercial ELISA kits. The protein and lipid content of the whole carcasses was determined. Data was analyzed using the MIXED procedure in SAS. High MEI pullets had 2.3-fold higher GnRH and 1.8-fold higher GnRH-RI mRNA levels than Low MEI pullets. MEI affected neither expression of GnIH and

GnIH-R genes, nor carcass protein content. From 22 to 26 wk of age ($P < 0.05$); LH, FSH, E2 concentrations, and carcass lipid content were 1.9, 1.6, 1.6, and 1.3% times higher respectively for High MEI (489 kcal/d) compared to Low MEI (258 kcal/d). The onset of lay for High MEI was advanced such that 100% had laid by 26 wk of age, compared to 30% in Low MEI. We concluded that High MEI advanced activation of the hypothalamic-pituitary-gonadal axis, increased body lipid deposition, and stimulated reproductive hormone levels which overall accelerated puberty in broiler breeder pullets.

Preface

Chapter 3 of this thesis has been published as S. H. Hadinia, P. R. O. Carneiro, C. A. Ouellette, and M. J. Zuidhof (2018) "Energy partitioning by broiler breeder pullets in skip-a-day and precision feeding systems" in Poultry Science**,** 97:4279–4289. I performed laboratory analysis, managed and analyzed data, and drafted the manuscript. M. J. Zuidhof secured funding, participated in experimental design and contributed to editing of the manuscript. C. A. Ouellette helped develop, and provided daily technical support for the precision feeding system. P. R. O. Carneiro assisted with data and sample collection. All co-authors read and approved the manuscript.

Chapter 4 of this thesis has been accepted for publication as S. H. Hadinia, P. R. O. Carneiro, D. R. Korver, and M. J. Zuidhof "Energy partitioning by broiler breeder hens in conventional daily restricted feeding and precision feeding systems" in Poultry Science. I collected samples and run laboratory analyses. I managed and analyzed data and also drafted the manuscript. M. J. Zuidhof conceived the study, designed the experiment, and contributed to the editing of the manuscript. D. R. Korver formulated the rations and participated in manuscript revisions. P. R. O. Carneiro assisted with data and sample collections at the poultry unit. All co-authors read and approved the manuscript.

Chapter 5 of this thesis has been submitted as S. H. Hadinia, P. R. O. Carneiro, C. J. Fitzsimmons, G. Y. Bédécarrats, and M. J. Zuidhof "Post photostimulation energy intake accelerated pubertal development in broiler breeder pullets" in Poultry Science. I performed sample analysis, managed and analyzed

data, and drafted the manuscript. M. J. Zuidhof designed the experiment and contributed to editing of the manuscript. G. Y. Bédécarrats provided technical support for sample collections, laboratory analyses, interpreting the laboratory results, and also offered editorial suggestions. C. J. Fitzsimmons provided technical support for the laboratory analyses and offered editorial suggestions as well. P. R. O. Carneiro participated in data and sample collections at the poultry unit. All co-authors read and approved the manuscript.

Dedication

"This thesis is dedicated to my parents and my sister, who have always encouraged me to pursue my academic goals"

Acknowledgments

First and foremost, I would like to thank my advisor, Dr. Martin Zuidhof, for providing me with the opportunity to join his group. You enhanced my passion for poultry research, as you provided guidance and encouragement in pursuit of my PhD degree. I would never have been able to finish my PhD program without your guidance. I appreciate the countless times you gave me advice while helping me overcome the challenges I encountered throughout my program. Your vision, patience, kindness, support, and confidence that you bestow upon me are greatly appreciated. I am proud of being your PhD student and working under your supervision. You provided me with the opportunity to develop my skills in the lab and learn by my mistakes. I still remember you told me there is nothing wrong with making mistakes, just taking a few deep breaths, double checking the steps you need to take, and just taking your time can help you calm down and avoid major errors. You also taught me how to practice writing and thinking within a good structure and you enhanced my scientific writing skills by revising my writings and encouraging me to pay attention to how professional scientists write about their work. You have helped me to enhance my modeling skills way more than I thought. You helped me to become a stronger person compared to when I started my PhD program. I have grown up, I think differently, I do things differently, my logic is different. I am a happier person because you saw the potential in me and helped me a lot to develop my professional skills. Thanks again for everything you have done for me.

Secondly, I would like to thank my advisory committee members, Dr. Doug Korver for helping me understand and expand my knowledge on the nutritional aspects of my research program and also for helping to improve my scientific writing. I sincerely appreciate the time and patience you had with me. I would like to also thank Dr. Carolyn Fitzsimmons for helping me understand and expand my knowledge on molecular biology aspects of my program. Your excellent guidance, caring, and scientific support during my PhD program has helped me a lot. You always had an open door for me regardless of how busy you were. Your help, kindness and encouragement throughout my program are much appreciated. I also thank Dr. Bruce Rathgeber and Dr. John Basarab for accepting to serve as external examiners for my thesis defense.

I would also like to thank Dr. Grégoy Bédécarrats from University of Guelph, for the excellent guidance, and scientific support for reproductive physiological aspect of my program. Your guidance and support with sending me all training videos for collecting hypothalamus and pituitary samples, laboratory protocols have helped a lot to improve my understating of reproductive physiology. Without your guidance I would never have been able to accomplish the reproductive physiology aspect of my program. I greatly appreciate your time, patience, kindness, and encouragement throughout my program.

I would like to thank all staff of the Poultry Research Center especially Shawn Rankin who always ensured that birds were well taken care of. I would also like to thank Lyle Bouvier, the previous Poultry Research Center manager who always provided me with the technical support and I really appreciate your patience

and kindness. I would like to extend my sincere thanks to Chris Ouellette, for providing me with the technical support and training me on working with precision feeding system. Your continued interest and sense of humor helped me a lot throughout my trials to cope with stress and I am very grateful for your help.

I would especially like to thank my colleague and friend, Paulo Roberto De Oliveira Carneiro, for your help and support and the countless hours you spent helping me at the Poultry Research Center. I was lucky for having you around myself and I greatly appreciate you for always willing to help, give your best suggestions, and for all the fun we have had. As well, thank you to all graduate and undergraduate volunteers and research assistants who have helped me with data collection.

To my family and friends, thank you for all the love and support. I am grateful for my parents for being supportive in my decision to pursue my abroad PhD studies. Thank you so much for all hours worked and money saved to help me. And finally, God who is always there for me and I will give thanks to you, LORD, with all my heart.

Last but not least, I acknowledge and greatly appreciate financial support from the following institutions and organizations: the Alberta Agriculture and Forestry (Edmonton, Alberta, Canada), Alberta Innovates Bio Solutions (Edmonton, Alberta, Canada), Agriculture and Food Council (Edmonton, Alberta, Canada), Alberta Chicken Producers (Edmonton, Alberta, Canada), the Poultry Industry Council (Guelph, Ontario, Canada), Danisco Animal Nutrition (DuPont; Marlborough, Wiltshire, United Kingdom), Canadian Hatching Egg Producers

(Ottawa, Ontario, Canada), Alberta Hatching Egg Producers (Edmonton, Alberta, Canada), and the Ontario Broiler Chicken Hatching Egg Producers Association (Guelph, Ontario, Canada) is gratefully acknowledged. In kind support for the precision feeding system was provided by Xanantec Technologies, Inc. (Edmonton, Alberta, Canada).

Table of Contents

Abstract ii

Preface v

Acknowledgments viii

Table of Contents xii

List of Tables xxi

List of Figures xxiv

List of Abbreviations xxv

1 Chapter 1. General Introduction 1

1.1 Introduction 1

1.2 References 3

2 Chapter 2. Literature Review 6

2.1 Broiler Breeder Challenges 6

2.2 Management Strategies of Broiler Breeders 7

2.2.1 Quantitative Feed Restriction Programs 9

2.2.2 Qualitative Feeding Restriction Programs 11

2.2.3 Feed Allocation 11

2.2.4 Precision Feeding System for Broiler Breeders 12

2.3 Broiler Breeder Metabolism 14

2.3.1 Storage and Mobilization of Nutrients 14

2.3.2 Feeding Frequency 15

2.4 Feed Efficiency and Energy Efficiency 17

2.4.1 Feed Efficiency 17

2.4.2 Energy Efficiency 17

2.5 Energy, Different Energy Systems and Energy Requirement in Poultry .. 19

2.5.1 Utilization of Dietary Energy 19

2.5.2 Energy Partitioning 20

2.6 Endocrinology and Reproduction 22

2.6.1 Regulation of Feed Intake by the Central Nervous System 22

2.6.2 Neuroendocrine Control of Reproduction 24

2.6.3 Age, Body Weight, and Body Composition at Sexual Maturation 26

2.7 Conclusion 28

2.8 Objectives 29

2.9 Hypotheses 30

2.10References 31

3 Chapter 3. Energy Partitioning by Broiler Breeder Pullets in Skip-a-day and Precision Feeding Systems 45

3.1 Abstract 45

3.2 Introduction 46

3.3 Materials and Methods 48

3.3.1 Experimental Design 48

3.3.2 Precision and Conventional Feeding Treatments 49

3.3.3 Management 51

3.3.4 Diet Composition 51

3.3.5 Carcass Traits 52

3.3.6 Statistical Analysis 52

3.3.6.1 Analysis of Variance 52

3.3.6.2 ME Partitioning Model 53

3.3.7 Residual Total HP 53

3.4 Results and Discussion 54

3.4.1 ME Intake 54

3.4.2 Growth 57

3.4.3 Carcass Traits 57

3.4.4 ME Partitioning Model 59

3.4.5 The ME Requirement for BW Gain 59

3.4.6 Total HP 60

3.4.7 Residual Total HP 61

3.4.8 Energetic Efficiency 62

3.4.9 Feed Conversion Ratio 64

3.5 Conclusions 64

3.6 Acknowledgments 65

3.7 References 65

3.8 Tables 73

3.9 Figures 80

4 Chapter 4: Energy Partitioning by Broiler Breeder Hens in Conventional Daily Restricted Feeding and Precision Feeding Systems 81

4.1 Abstract 81

4.2 Introduction 82

4.3 Materials and Methods 85

4.3.1 Experimental Design 85

4.3.2 Precision and Conventional Feeding Treatments 86

4.3.3 Management 87

4.3.4 Measurement of AME 87

4.3.5 Carcass Traits 88

4.3.5.1 Breast Muscle and Fat Pad 88

4.3.5.2 Oviduct and Ovarian Morphology 89

4.3.6 Age at 50% Production 89

4.3.7 Metabolizable Energy Partitioning Model 90

4.3.8 Partitioning of ME Intake to Growth, Total HP, and Egg Mass 90

4.3.9 Regression Analysis between ME Intake and Weekly Total HP 91

4.3.10 Egg Composition 91

4.3.11 Statistical Analysis 92

4.3.11.1 Analysis of Variance 92

4.4 Results and Discussion 93

4.4.1 Metabolizable Energy Intake, Body Weight, and Average Daily Gain 93

4.4.2 Carcass Traits 95

4.4.3 Sexual Maturation and Egg Production 96

4.4.4 Egg Weight and Egg Mass 98

4.4.5 Ovarian Morphology 98

4.4.6 Metabolizable Energy Partitioning 99

4.4.6.1 The ME Requirement for BW Gain 99

4.4.6.2 Total Heat Production 100

4.4.6.3 The ME Requirement for Egg Mass 103

4.4.7 Energetic Efficiency 104

4.5 Conclusions 105

4.6 Acknowledgments 107

4.7 References 107

4.8 Tables 116

4.9 Figures 124

5 Chapter 5: Post-photostimulation Energy Intake Accelerated Puberty in Broiler Breeder Pullets 125

5.1 Abstract 125

5.2 Introduction 126

5.3 Materials and Methods 130

5.3.1 Experimental Design 130

5.3.2 Diet Analyses 131

5.3.3 Management 132

5.3.4 Sample Collection 133

5.3.4.1 Dissection 133

5.3.4.2 Hypothalamus and Pituitary Tissue Collection 133

5.3.4.3 Blood Collection 134

5.3.4.4 Carcass Collection 134

5.3.5 Lab Analyses 134

5.3.5.1 RNA Isolation, cDNA Synthesis, and Real-time Quantitative PCR 134

5.3.5.2 Hormone Assay 137

5.3.5.3 Carcass Composition 138
5.3.6 Statistical Analysis 139
5.3.6.1 Pearson Correlation 139
5.3.6.2 Analysis of Variance 139
5.4 Results and Discussion 140
5.4.1 Metabolizable Energy Intake, and BW 140
5.4.2 Gene Expression 141
5.4.2.1 Effect of ME Intake on Genes of the Reproductive Axis 141
5.4.2.2 Effect of ME intake on Genes Involved in the Regulation of Energy Balance 144
5.4.3 The Correlation between the Genes 145
5.4.4 Reproductive Hormones 146
5.4.5 Carcass Composition 149
5.4.6 The Onset of Sexual Maturity 151
5.5 Conclusion 153
5.6 Acknowledgments 154
5.7 References 154
5.8 Tables 166
6 Chapter 6: Synthesis 176
6.1 General Discussion 176

6.2 Novelty of Research 183

6.3 Study Limitations 184

6.4 Future Research 187

6.4.1 Genes Involved in the Regulation of Energy Balance 188

6.4.1.1 Agouti-related Peptide 188

6.4.1.2 Liver Kinase B1, Mouse Protein MO25, and STE20-related Adaptor Protein 188

6.4.1.3 Leptin 189

6.4.1.4 Melanocyte Stimulating Hormone Alpha and Melanocortin Receptor Type 4 190

6.4.1.5 Corticotropin Releasing Hormone 190

6.4.2 Genes Involved in the Regulation of Energy Balance and Reproductive Axis 191

6.4.2.1 Thyroid-stimulating Hormone 191

6.4.2.2 Growth Hormone, and Growth Hormone Receptor 192

6.5 Overall Implications 193

6.6 Conclusion 194

6.7 References 195

6.8 Figures 202

References 206

Appendix (Supplementary Materials) 239

Hormonal Analyses 239

Plasma LH and FSH Analyses 239

Plasma E2 Analysis 240

List of Tables

Table 3-1. Composition and calculated analysis of broiler breeder pullet grower diet provided from 10 to 23 wk of age. 73

Table 3-2. Metabolizable energy intake (MEI) of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age. 74

Table 3-3. Body weight of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age..... 75

Table 3-4. Average daily gain of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age..... 76

Table 3-5. Daily total heat production (HP), residual feed intake (RFI), residual daily total heat production (RHP), and proportion of expected ME intake (MEI) partitioned to HP and growth by breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age..... 77

Table 3-6. Proportional breast muscle and abdominal fat pad weights of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age. 78

Table 3-7. Cumulative feed conversion ratio of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age. 79

Table 4-1. Composition and calculated analysis of broiler breeder diet provided from 23 to 34 wk of age. 116

Table 4-2. Metabolizable energy intake (MEI), average daily gain (ADG), and body weight (BW) of broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age. 117

Table 4-3. Carcass characteristics in broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments at 23, 28, 32, and 34 wk of age. 118

Table 4-4. Hen-day egg production, and egg weight of broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age. 119

Table 4-5. Carcass characteristics in broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments at 23, 28, 32, and 34 wk of age. 120

Table 4-6. Large yellow follicle numbers (LYF), hierarchical follicle weight, hierarchical follicle diameters of breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments at 34 wk of age. 121

Table 4-7. Total heat production (HP), growth and egg mass (EM), and residual feed intake (RFI) of broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age. 122

Table 4-8. The coefficient of age-specific (A; weekly) total heat production (HP) and the total HP ($A \times BW^{0.67}$) of breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age. 123

Table 5-1. Composition of broiler breeder pullet grower diets for low ME intake (Low MEI) and high ME intake (High MEI) treatments 166

Table 5-2. Sequences of the oligonucleotide primers used in quantitative real-time PCR, and the publicly available sequences they were designed from 167

Table 5-3. Body weight (BW), ME intake (MEI), and percentage of flock in lay for broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 28 wk of age 168

Table 5-4. Relative expression of gonadotropin releasing hormone (GnRH-I) and gonadotropin inhibitory hormone (GnIH) in hypothalamus and GnRH-I receptor-I (GnRH-RI), GnRH-I receptor-III (GnRH-RIII), GnIH receptor (GnIH-R) genes in anterior pituitary of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age..... 169

Table 5-5. Relative expression of luteinizing hormone (LH) and follicular stimulating hormone (FSH) genes in anterior pituitary and neuropeptide Y (NPY), proopiomelanocortin (POMC), and leptin receptor (LEPR), genes in hypothalamus of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age..... 171

Table 5-6. The ratios of relative expression of gonadotropin releasing hormone (GnRH-I) receptor-I (GnRH-RI), GnRH-I receptor-III (GnRH-RIII), and gonadotropin inhibitory hormone receptor (GnIH-R) genes in anterior pituitary of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age..... 173

Table 5-7. Plasma concentrations of luteinizing hormone (LH), follicular stimulating hormone (FSH), and 17 beta-estradiol (E2) of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age..... 174

Table 5-8. Proportional carcass composition of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age..... 175

List of Figures

Figure 3-1. Regression of pen-specific total heat production [E(a + u)] versus ME intake from 10 to 23 wk of age 80

Figure 4-1. Regression of average total heat production [A] versus ME intake from 23 to 34 wk of age..... 124

Figure 6-1. Comparison of estimated total heat production (HP, maintenance requirement) among the current study, Sakomura et al., (2003), and Pinchasov and Galili (1990) for broiler breeders 202

Figure 6-2. Comparison of body fat composition in broiler breeders among the current study (2019, Chapter 4), Bennett and Leeson (1990), Miles et al. (1997); Renema et al. (2007a); van Emous et al. (2015); and Zuidhof (2018). 203

Figure 6-3. The hypothalamic-pituitary-gonadal axis (HPG axis), the hypothalamus-pituitary-adrenal axis (HPA axis), the hypothalamic-pituitary-thyroid axis (HPT axis), and the hypothalamic-pituitary-somatotropic axis (HPS axis) in broiler breeders 205

List of Abbreviations

ACTB	Beta-actin
ACTH	Adrenocorticotropic hormone
ADG	Average daily gain
AGRP	Agouti-related peptide
AMPK	Adenosine monophosphate-activated protein kinase
BW	Body weight
CON-skip-a-day	Conventional skip-a-day feeding
CON-daily restricted	Conventional daily restricted feeding
CNS	Central nervous system
CRH	Corticotropin releasing hormone
E2	Estradiol
EEF1A1	Eukaryotic translation elongation factor 1 alpha 1
EM	Egg mass
FCR	Feed conversion ratio
FSH	Follicle stimulating hormone
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GE	Gross energy
GH	Growth hormone
GH-R	GH receptor
GnIH	Gonadotropin inhibitory hormone
GnIH-R	Gonadotropin inhibitory hormone receptor
GnRH	Gonadotropin releasing hormone

GnRHR-I	Gonadotropin releasing hormone receptor-I
GnRHR-III	Gonadotropin releasing hormone receptor-III
HDEP	Hen-day egg production
HPA	Hypothalamus-pituitary-adrenal
HPG	Hypothalamic–pituitary–gonadal
HPT	Hypothalamus-pituitary-thyroid
ICV	Intra-cerebroventricular
IGF-I	Insulin-like growth factor-I
IGF-I-R	Insulin-like growth factor-I-receptor
LEPR	Leptin receptor
LKB1	Liver kinase B1
LH	Luteinizing hormone
LYF	Large yellow follicle
ME	Metabolizable energy
MEI	Metabolizable energy intake
ME_m	Metabolizable energy requirements for maintenance
MCR-4	Melanocortin receptor type 4
MO25	Mouse protein MO25
α-MSH	Melanocyte stimulating hormone alpha
RME_m	Residual maintenance Metabolizable energy requirement
ME_g	Metabolizable energy cost for BW gain
NPY	Neuropeptide Y
PF	Precision feeding system

POMC	Pro-opiomelanocortin
RFI	Residual feed intake
RFID	Radio frequency identification
RHP	Residual total heat production
RPL19	Ribosomal protein L19
SDHA	Succinate dehydrogenase complex flavoprotein subunit A
STRAD	STE20-related adaptor protein
TRH	Thyrotropin releasing hormone
TSH	Thyroid-stimulating hormone

1 Chapter 1. General Introduction

1.1 Introduction

Commercial selection programs for rapid growth and feed efficiency in broiler chickens have been successful; however, this has come at the expense of reproductive efficiency in broiler breeders (Decuypere et al., 2010; Pollock, 1999). Broiler breeders are feed restricted because if broiler breeders fed *ad libitum* they would become overweight and lay significantly fewer eggs and develop health issues such as prolapsed cloaca, culling from lameness, and sudden death (Renema et al., 1999a; Yu et al., 1992a). Thus, numerous studies have been conducted to identify optimal feed restriction practices for broiler breeders.

According to Zuidhof et al. (2015), pullets in a skip-a-day feeding treatment had lower breast muscle weight and had higher fat pad weight compared to pullets in daily restricted treatments at 22 wk of age. The abdominal fat pad is more efficient for storage and mobilization of nutrients and conditioned to repeated energy shortage, and skip-a-day fed birds compromised growth of their breast muscle and diverted more energy to storage in their abdominal fat pad (Zuidhof et al., 2015). On the other hand, limited daily feeding increased efficiency compared to skip-a-day feeding due to reduced storage and mobilization of nutrients (de Beer and Coon, 2007; Zuidhof et al., 2015). Carcass composition (Nonis and Gous, 2016; Renema et al., 1999a), body weight (**BW**; van der Klein et al., 2018a), hypothalamic maturation (age; Bédécarrats et al., 2016), concentration of reproductive hormones (Renema et al., 1999b), photostimulation (van der Klein et al., 2018b), and feed intake levels (Renema et al., 1999a) can affect onset of lay in broiler breeders. Laying hens with greater BW and greater lipid content entered into lay earlier than birds with lower BW and lower lipid content (Summers and Leeson, 1983). A new method of feeding is required to provide all metabolic triggers for the onset of sexual maturation and increase egg

production during laying period while also increases efficiency. A precision feeding system for broiler breeders was developed at the University of Alberta to provide right amount of feed to each bird, increase BW uniformity, reduce storage and mobilization of nutrients, and increase efficiency by providing several small meals in a day. Moreover, the PF system was developed to increase egg production by providing metabolic triggers for the onset of sexual maturation such as BW, adequate body fat and body protein contents. Eitan et al. (2014) reported that broiler breeder hens in 2000 had lower abdominal fat pad (2.67%) compared to hens in 1980 (5.37%) and sexual maturation was delayed by 28.3 d. This could be due to increasing the degree of feed restriction of broiler breeders in 2000 compared to 1980. The degree of feed restriction in broiler breeders has become more severe because their growth potential has increased during the last 30 years (van Emous, 2015; Renema et al., 2007) whereas the target BW for broiler breeders remained almost constant (Renema et al., 2007; Hadinia et al., 2018). Hadinia et al. (2018) reported that average daily gain (**ADG**) was 15 g for broiler breeder pullets from 10 to 23 wk of age which was almost similar to the values reported by Sakomura et al. (2003) and Pinchasov and Galili (1990). Sakomura et al., 2003 reported the values of 13 and 16 g/d for broiler breeder pullets from 9 to 14 and 15 to 20 wk of age, respectively (Sakomura et al., 2003). Pinchasov and Galili, 1990 reported the value of 14 g/d from 3 to 20 wk of age. Apparently, the target BW of broiler breeders have undergone little change from 1990 to 2018. The gap between growth potential of broilers and broiler breeder target BW is increasing (Zuidhof, 2018). Renema et al. (1999a,b) reported that an *ad libitum* feeding regimen increased luteinizing hormone and follicle stimulating hormone production and advanced the onset of lay by 13.6 d compared to restricted feeding in broiler breeder hens. This suggested that energy balance may interact with BW and age threshold in the initiation of sexual maturation after photostimulation (Renema et al., 1999b). Although these

studies have provided significant information regarding different factors that can affect the onset of sexual maturation in broiler breeders, very limited information is available about the effect of energy intake on BW, hypothalamic maturation (age), reproductive hormones, carcass composition, and onset of sexual maturation. Thus, new research is required in this regard. Moreover, it is also important to compare the effects of conventional feeding methods with the new precision feeding system, on performance, efficiency, and reproduction in broiler breeders.

The following review discusses the literature to establish the state of knowledge in the research area and identify key methodological issues (Chapter 2). Energy partitioning, carcass traits, feed and energetic efficiency in broiler breeder pullets were assessed using a conventional skip-a-day feeding program and a precision feeding system (Chapter 3). Moreover, energy partitioning, carcass traits, age at 50% production, and energetic efficiency in broiler breeder hens were evaluated in a daily restricted feeding program and a precision feeding system (Chapter 4). Additionally, the effect of energy intake in broiler breeder pullets was assessed on carcass compositions, reproductive hormones, onset of sexual maturation, genes involved in reproduction axis and genes related to energy balance (Chapter 5). Lastly, main outputs of the experimental objectives for both the industry and science are discussed in Chapter 6. Moreover, the novelty of the projects in the current thesis, the limitations for the projects in the current thesis, the overall implications, and proposed future research are discussed in Chapter 6.

1.2 References

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927–1939.

de Beer, M., R. W. Rosebrough, B. A. Russell, S. M. Poch, M. P. Richards, and C. N. Coon. 2007. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. Poult. Sci. 86:1726–1738.

Decuypere, E., V. Bruggeman, N. Everaert, Y. Li, R. Boonen, J. de Tavernier, S. Janssens, and N. Buys. 2010. The broiler breeder paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. Br. Poult. Sci. 51:569–579.

de Jong, I. C., B. Jones. 2006. Feed restriction and welfare in domestic birds. Pages 120-135 in Feeding in Domestic Vertebrates. Wallingford, United Kingdom.

Eitan, Y., E. Lipkin, and M. Soller. 2014. Body composition and reproductive performance at entry into lay of anno 1980 versus anno 2000 broiler breeder females under fast and slow release from feed restriction. Poult. Sci. 93:1227–1235.

Hadinia, S. H., P. R. O. Carneiro, C. A. Ouellette, and M. J. Zuidhof. 2018. Metabolizable energy partitioning of broiler breeder pullets in skip-a-day and precision feeding systems. Poult. Sci. 97:4279–4289.

Pollock, D. L. 1999. A geneticist's perspective from within a broiler primary breeder company. Poult. Sci. 78:414–418.

Renema, R. A., F. E. Robinson, M. Newcombe, and R. I. Mckay. 1999a. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 1. Growth and carcass characteristics. Poult. Sci. 78:619–628.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I McKay. 1999b. Effect of body weight and feed allocation during sexual maturation in broiler breeder hens. 2. Ovarian morphology and plasma hormone profiles. Poult. Sci. 78:629–639.

Renema, R. A., M. E. Rustad, and F. E. Robinson. 2007. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. World's Poult. Sci. J. 63:457–472.

Savory, C. J., K. Maros, and S. M. Rutter. 1993. Assessment of hunger in growing broiler breeders in relation to a commercial restricted feeding program. Anim. Welf. 2:131–152.

Summers, J. D., and S. Leeson. 1983. Factors influencing early egg size. Poult. Sci. 62:1155–1159.

van Emous, R. 2015. Body composition and reproduction in broiler breeders: impact of feeding strategies. PhD Diss. Univ. Wageningen, Wageningen, the Netherland.

Yu, M., F. E. Robinson, and A. R. Robblee. 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. Poult. Sci. 71:1739–1749.

Zuidhof, M. J. 2018. Lifetime productivity of conventionally and precision-fed broiler breeders. Poult. Sci. 97:3921–3937.

Zuidhof, M. J., D. E. Holm, R. A. Renema, M. A. Jalal, and F. E. Robinson. 2015. Effects of broiler breeder management on pullet body weight and carcass uniformity. Poult. Sci. 94:1389–1397.

2 Chapter 2. Literature Review

2.1 Broiler Breeder Challenges

For broiler chickens, which are offspring of broiler breeders, intensive genetic selection for rapid growth and feed efficiency has been successful. An unintended consequence of these selection programs for broiler breeder stocks was decreasing body fat deposition that delayed the onset of lay. At 22 wk of age, abdominal fat pad weight (% BW) was 3.60% using a standard diet in Hubbard broiler breeders (Bowmaker and Gous, 1989). It was reported that at 22 wk of age, abdominal fat pad weight (% BW) was 2.72% in Arbor Acres broiler breeders fed a standard diet (Fattori et al., 1993). Renema et al. (2001) reported that at 21 wk of age, abdominal fat pad weight (% BW) was 1.54% using a standard diet in Shaver Starbro broiler breeder pullets. Hadinia et al. (2018) reported that abdominal fat pad weight (% BW) was 1.45% at 23 wk of age using a standard diet in Ross 308 broiler breeder pullets. For comparative purposes, only data from studies of broiler breeders at the onset of lay (between wk 21 and 23) and a BW between 2,336 and 2,648 g were used. Moreover, the ratio of energy to protein in the studies by Bowmaker and Gous (1989), Renema et al. (2001), Hadinia et al. (2018), and Fattori et al. (1993) were respectively 1.87, 1.81, 1.87, and 1.90. The feed intake at 22 wk of age, in the studies by Bowmaker and Gous (1989), Renema et al. (2001), and Hadinia et al. (2018) were respectively 148, 93, and 97 g/bird/d. However, Fattori et al. (1993) did not report any information about the feed intake of the birds at 22 wk of age. These results indicated that feed allocation for broiler breeders has been reduced by 34% from 1989 to 2018. It seems that the decrease in the abdominal fat pad weight was due to the increase in feed restriction level. The abdominal fat pad weight (% BW) at the end of the rearing period has declined by 2.48% in 29 years from 1989 to 2018. The weight of abdominal fat pad is linked directly to total body fat content in avian species thus it is a reliable indicator for judging

total body fat content (Thomas et al., 1983; Becker et al., 1979). Decreasing carcass fat in meat-type pullets compared with 30 years ago brings up concerns that excessive leanness in meat-type broiler breeders may negatively affect reproductive success (Zuidhof., 2018). Broiler breeder hens from a line unselected since 2000 and 2.65% of abdominal fat pad delayed onset of laying 19.2 d compared with hens from a line unselected since 1980 and 5.38% of abdominal fat pad using a standard diet (Eitan et al., 2014). van Emous (2015) reported 0.44% of abdominal fat pad at 20 wk of age for broiler breeder pullets. Looking back through the literature over time, it is obvious that fat deposition has been decreasing in pre-pubertal meat-type pullets and broiler breeders are getting much leaner (van Emous., 2015).

Body fat content affects yolk synthesis because yolk synthesis in the birds is regulated by incorporation of lipids originating from the diet, lipoproteins from the liver, and release of lipids from the adipose tissue (Yang et al., 2013). Thus, the reduction in fat deposition in the body can reduce egg production in broiler breeders.

This chapter will focus on different aspects of broiler breeders to establish the state of knowledge in management strategies such as different feeding systems, changes in metabolism by different feeding systems; the difference of feed and energetic efficiency, energy requirements and energy partitioning, and endocrinology, thresholds for sexual maturation, and reproduction to identify key methodological issues.

2.2 Management Strategies of Broiler Breeders

Broiler breeders are feed restricted, otherwise they grow rapidly and become overweight and suffer from a number of health problems such as obesity resulting in high mortality if they are fed to appetite (Renema and Robinson, 2004; Heck et al., 2004). It was reported that broiler breeders fed *ad libitum* increased rates of mortality (Heck et al., 2004; Williams, 2012) and reduced

settable egg production (Mohiti-Asli et al., 2012; Onagbesan et al., 2006; Hocking et al., 2002; Yu et al., 1992), fertility (Mohiti-Asli et al., 2012; Hocking et al., 2002; Goerzen et al., 1996; Yu et al., 1992) and hatchability (Mohiti-Asli et al., 2012; Hocking et al., 2002; Yu et al., 1992). From approximately 14 wk of age, overfeeding resulted in excess growth in broiler breeders, production of excess ovarian follicles and consequently decreased reproduction performance (Renema et al., 1999; Wei et al., 2019). Overfeeding results in the formation of excess large yellow ovarian follicles, which are likely arranged in multiple hierarchies of large follicles (Yu et al., 1992; Johnson, 2012; Williams, 2012). Thus broiler breeders are feed restricted to optimize their reproductive performance. On the other hand, fat deposition in broiler breeders has decreased compared with 29 years ago (as explained in section 2.1) and leanness in broiler breeders may negatively affect reproductive success (Zuidhof., 2018). It was recently suggested that a relaxation in feed restriction could increase fat pad deposition and egg production in broiler breeder pullets (Zuidhof, 2018).

In broiler breeders feed restriction starts by 1 to 3 wk of age (Mench, 2002). The period of most severe feed restriction in broiler breeders occurs between 8 to 16 wk of age, during where feed intake is between 25 to 33% of the intake of *ad libitum* fed pullets (de Jong, 2006) although no recent data are available. Feed restriction is less severe during the laying period and feed intake is between 50 to 90% of the intake of *ad libitum* fed hens (Bruggeman et al., 1999; Hocking et al., 2002) although, also here no recent data are available. To indirectly measure the degree of hunger in feed restricted broiler breeders, behavioural and physiological indices are assessed as indicators (de Jong and Guemene, 2011; Tolkamp and D'Eath, 2016). Frustration, related to feeding motivation, is reflected in oral behaviors (drinking, foraging and pecking), and thus indirectly illustrates the degree of hunger in poultry species (Savory et al., 1993; de Jong and Guemene,

2011; Gilmet, 2015). Feed restricted broiler breeders increased oral behaviors such as drinking, foraging, and spot pecking compared to those fed *ad libitum* (Hocking et al., 2006; Merlet et al., 2005; Jones et al., 2004). In contrast, comfort and resting behaviours were reduced in feed-restricted breeders (Merlet et al., 2005; Jones et al., 2004; Morrissey et al., 2014). Feed restriction causes feed competition between broiler breeders and birds that are more aggressive consume more feed than less aggressive birds and this reduces flock uniformity (Zuidhof et al., 2015). Thus choosing a suitable feed restriction program for broiler breeders is important. There are different methods for feed restriction of broiler breeders, which will be addressed below.

2.2.1 Quantitative Feed Restriction Programs

Limited everyday feed restriction and skip-a-day feeding programs are the most practical, and therefore commonly used by the broiler breeder industry. In limited everyday feed restriction, a restricted amount of feed is given to broiler breeders daily whereas, in a skip-a-day program, a feed allotment for two days is fed to broiler breeders every second day. When feed intake rate of the most aggressive birds slows or they leave the feeder, the amount of feed remaining is sufficient to allow less aggressive birds to receive an adequate amount of feed. Skip-a-day feeding is often preferred to limited daily feeding because it increases flock uniformity (Cobb-Vantress, 2005). Because the feed allotment on feeding days in a skip-a-day feeding program is more than with a limited daily feeding program, feed cleanup time is increased, which allows for more equal distribution of feed intake and a more uniform flock (de Beer and Coon, 2007). Zuidhof et al (2015) reported that breeder uniformity in skip-a-day feeding was increased by 1.20 times at 22 wk of age compared to daily feeding ($P < 0.05$) however uniformity did not differ between skip-a-day and daily feedings at 3, 7, 11, 15 and 19 wk of age. In contrast, daily-fed broiler breeders were more efficient compared to skip-a-day birds due to the longer duration of the supply of nutrients

(de Beer et al., 2007; Zuidhof et al., 2015). Another feed restriction method is 4/3 feeding program (four days fed and 3 not consecutive days off feed per week) and 5/2 feeding program (five days fed and 2 not consecutive days off feed per week). The 4/3 and 5/2 feeding programs allow farmers to feed the birds on the same days of the week which make a consistent weekly program and a good solution to facilitate simpler management during the weekends (Cobb-Vantress, 2008). It is important to note that in limited daily, skip-a-day, 4/3 and 5/2 feed restriction programs the amount of allocated feed in a week will be the same although the number of days on which they are fed are different.

de Beer and Coon (2007) reported that hens fed on daily restricted during rearing and laying periods eventually produced more eggs than broiler breeder fed on skip-a-day (fed on alternate days) program during rearing period and switched to daily feeding program on laying period. This was probably due to more frequent availability of dietary nutrients in a daily-restricted feeding program since deposition and mobilization of nutrients are not completely efficient processes (de Beer and Coon, 2007). This result was in consistent with Hadinia et al. (2019), who reported that broiler breeders on a skip-a-day feeding program during the rearing period and a daily restricted feeding program during the laying period had higher egg production than hens fed daily restricted and receiving several small meals per day. On the other hand, broiler breeder hens fed on a 5-2 feeding program (feeding on 5 d/wk) during the rearing period and switched to a daily feeding program during the laying period had larger eggs than hens which were fed on a daily restricted feeding program during the rearing and laying periods (de Beer and Coon, 2007). Metabolic changes such as increased lipogenesis may result in larger eggs in feeding programs that include off-feed days (de Beer and Coon, 2007).

2.2.2 Qualitative Feeding Restriction Programs

The qualitative feed restriction program is another feed restriction method to control body weight (**BW**) of broiler breeders. It involves using a dietary dilution by changing diet composition which results in dilution of nutrients per kg of diet. Some ingredients such as sugar beet pulp, sawdust, or oat hulls can be used as diet diluents. Zuidhof et al. (1995) reported that the optimal level of nutrient dilution with ground oat hulls for broiler breeders was 15% of the total feed mass (adding 15 kg of ground oat hull to 85 kg of standard diet). This level increased productivity of the birds relative to the control treatment or to a diet composed of 30% of ground oat hulls. By adding diluents diet may help birds feel satiated because of gut fill and consequently may experience less stress (Mench, 2002). However, diluents can result in impaction in the digestive tract, result in producing additional excreta and may have adverse effects on production (Leeson and Summers, 1991; Savory et al., 1996). Zuidhof et al. (2015) demonstrated that broiler breeder pullets fed a standard mash diet on a skip-a-day feeding program were more efficient (FCR = 5.02) than birds fed with high fiber mash diet containing 25% lower nutrient density (FCR = 5.51). The lower ME content of the high fiber diet increased feed intake for those birds to meet their daily ME requirements.

2.2.3 Feed Allocation

Feed allocation decisions are difficult yet critical for managing BW of broiler breeders. The rationale for feed allocation decisions includes matching actual BW of broiler breeders with a pre-defined target BW. Schneider et al. (2005) explained how feed allocation decisions could be accurately made for broiler breeders. Ideally, frequent and accurate BW measurements would be used to calculate BW gain. If birds are gaining as expected, slightly increasing feed allocation is suggested, and if birds are gaining fast, maintaining feed allocation at the same level as the

previous week is suggested. Feed allocation decisions can affect flock uniformity and in a uniform flock, producers can better match the nutrient requirements of individual birds with feed allocation which leads to high peak production (Pishnamazi et al., 2008). Feed allocation can be adjusted for anticipated changes in egg production rates (Aviagen, 2018). Afterward, during post-peak production which usually occurs after 32 wk of age, the main goal of feed allocation is to maintain egg production as close to the peak as possible. Feed allocation in this period focuses on decreasing feed intake because the natural decrease in egg production reduces nutrient requirements. Moreover, decreasing feed intake reduces cost of feed and decreases the probability of obesity in broiler breeders. Precise feed allocation requires weighing birds frequently, which requires labor (Schneider et al., 2005) or automated weighing equipment. Both of these increase the cost of hatching egg production. Although a major concern with automated data systems is the cost, it is conceivable that automation might eventually increase profitability through increased chick production.

2.2.4 Precision Feeding System for Broiler Breeders

An automated precision feeding (**PF**) system was developed at the University of Alberta (Zuidhof et al., 2016, 2017) to weigh broiler breeders individually and allocate feed based on individual BW in real time. To use the PF system each bird is identified by a unique radio frequency identification tag. Every individual free run broiler breeder is weighed by a built-in platform scale when it enters the PF station. If its BW is equal to or greater than the target BW, the bird is gently ejected by the station. Target BW is interpolated hourly for the PF birds. On the other hand, if BW of a bird is lower than the target BW, the PF station provides access to approximately 25 g of feed for 1 min, after which the bird is ejected from the station. The amount of feed provided for birds and the duration of the feed access can vary according to the

requirements of each individual. A feeder mounted on a load cell weighs the feed before and after feeding. Feed intake for each feeding bout is calculated as the initial minus the final feed weight. After weighing, the feeder is topped up to provide approximately 25 g of feed for the next feeding bout. After each visit, radio frequency identification, BW, and initial and final feed weight data is recorded to a database with a date and time stamp. A monochromatic green LED light (525 nm wavelength) is mounted above the entry door and the feeder with a light intensity of 1.9 lux at the position of the feeder. The wavelength is strategically chosen to help birds to enter the feeding stations and see feed during the scotophase without stimulating hypothalamic photoreceptors (Rodriguez, 2017). Green light promoted early growth while using constant red supplemental light treatment delayed onset of lay in broiler breeder pullets for 2 to 3 wk due to a form of photorefractoriness (Rodriguez, 2017). Thus, the green LED light was used for the PF birds access to feed for 24 h/d and sequentially receive several small meals over a full 24 h/d rather than one large meal per day.

Zuidhof (2018) compared grandparent broiler breeder pullets fed by PF system fed on daily program. It was reported that PF pullets had lower BW CV (2%) compared to daily fed pullets (14%) at photostimulation (22 wk of age) due to feed allocations to individual birds based on their BW measured in real time. Each individual pullet in PF received on average 10 small meals throughout each day, compared with one larger meal per day in the daily feed restricted treatment that was fed on a group basis at once. PF pullets and daily fed pullets did not differ for fat pad weight at 22 wk of age, however, PF pullets had 1.2 times greater breast muscle weight than daily fed pullets at 22 wk of age. The PF treatment had 3.8% higher fertility compared with the daily fed hens. However, the PF hens laid 27% fewer eggs than the daily fed hens. This indicated that PF system did not provide a sufficient increase in nutrients before the onset of lay nor during the

laying period to help the PF birds to deposit enough fat and increase the egg production. Zuidhof (2018) explained that increased feeding frequency probably reduced diurnal fluctuations in nutrient supply and provided an insufficient metabolic trigger for sexual maturation. As explained previously, relaxing feed restriction in PF broiler breeders relative to conventionally-fed breeders may be necessary to increase fat deposition and egg production (Zuidhof., 2018).

2.3 Broiler Breeder Metabolism

2.3.1 Storage and Mobilization of Nutrients

Animals use feed as a source of nutrients to maintain their basal metabolism, physical activity, growth, and reproduction (Wang et al., 2006). Energy of feed is stored in fat, protein, and carbohydrate in birds (Blem, 1990; Guglielmo, 2018). Fat (triglycerides which can be stored in white adipose tissue) has more energy compared to protein and carbohydrate (Guglielmo, 2018). Adipose tissue contains 9.1 kcal/g energy (Johnston, 1970; Leeson and Summers, 2001; Romero et al., 2014), protein contains 5.5 kcal/g (Pullar and Webster, 1977; Leeson and Summers, 2001; Romero et al., 2014), and carbohydrate contains 4.1 kcal/g energy (Shils, 2006; Leeson and Summers, 2001; Romero et al., 2014). Glycogen is the main form of carbohydrate that animals store in their liver and muscle. Glycogen stores of birds are small and would meet energy demands for a short period (Blem, 1990).

When feed is not available for animals, such as during off-feed days during a feed restriction program, they need to mobilize stored nutrients for the body as energy sources (Wang et al., 2006). There are three metabolic phases (I, II, and III) occurring during feed deprivation in animals and each phase is characterized based on the primary energy source available as explained below (Wang et al., 2006).

- Phase I. The postabsorptive phase is the first phase of fasting and happens when the last meal has been absorbed from the gastrointestinal tract (Wang et al., 2006). During this period, which normally lasts for hours, glycogenolysis, or glycogen depletion of liver stores, maintain constant blood sugar levels (Wang et al., 2006). Glycogen converts to pyruvate via the glycolysis pathway and pyruvate eventually converts to acetyl-coA which produces energy through the Krebs cycle (Nelson and Cox, 2006). As phase II progresses, protein degradation decreases and degradation of adipose tissue begins to be used to meet the energy requirements (Wang et al., 2006).
- Phase II. When liver glycogen stores are depleted, gluconeogenesis supplies the energy requirements of organs (Wang et al., 2006). The initial fuel for gluconeogenesis is amino acids from proteolysis of muscle protein (Wang et al., 2006). Glucogenic amino acids are converted to glucose through gluconeogenesis and ketogenic amino acids can be degraded directly into acetyl-coA, which is the precursor of ketone bodies (Nelson and Cox, 2006). Moreover, glycerol which is liberated from adipose tissues is also another substrate for gluconeogenesis (Wang et al., 2006). Increased oxidation of fatty acids results in elevated production of ketone bodies which can be used as an oxidative fuel in many tissues including the brain (Wang et al., 2006). As phase II progresses, protein degradation is slow, and degradation of adipose tissue begins to be used to meet the energy requirements (Wang et al., 2006).
- Phase III. If starvation continues until the adipose tissue stores are depleted, muscle is rapidly degraded for further gluconeogenesis. The excessive loss of muscle protein eventually leads the animal death (Wang et al., 2006).

2.3.2 Feeding Frequency

Feed restriction method affects metabolism in broiler breeders. de Beer et al. (2007) assessed the effect of skip-a-day and daily feeding on metabolism in broiler breeder pullets. They

reported that genes involved in lipogenesis such as acetyl–coenzyme A carboxylase (ACC), malic enzyme (MAE), fatty acid synthase (FAS) were highly expressed 12 and 24 h after feeding in pullets fed using a skip-a-day program and these genes had smaller changes in daily-fed pullets. Additionally, isocitrate dehydrogenase (ICDH) and aspartate aminotransferase (AAT) had lower expression in skip-a-day pullets in refeeding and higher expression during fasting compared to the daily-fed pullets (de Beer et al., 2007). Both ICDH and AAT are indicative of gluconeogenesis. ICDH and AAT did not change in daily fed pullets. Gluconeogenesis was increased in skip-a-day pullets. Moreover, daily-fed pullets had more frequent nutrient supply relative to skip-a-day pullets. Thus, feeding birds using a skip-a-day feeding program would be less efficient because they need to continually store and mobilize nutrients (de Beer and Coon, 2007). However, metabolic changes such as increased lipogenesis may result in larger eggs in feeding programs that include off-feed days (de Beer and Coon, 2007). Broiler breeder hens fed on a 5-2 feeding program (feeding on 5 d/wk) during their rearing period had larger eggs during their laying period than hens fed on daily restricted feeding program during rearing and laying period (de Beer and Coon, 2007). Zuidhof (2018) demonstrated that more frequent feeding increased efficiency in grandparent broiler breeders, probably through allowing birds to utilize nutrients directly from the gastrointestinal tract and led to reduced storage and mobilization of nutrients in the body. This author hypothesized that decreased efficiency in daily fed grandparent broiler breeder hens was because those birds experienced more dramatic diurnal swings in energy balance compared to hens fed by the PF system. Moreover, daily fed hens were likely conditioned to store nutrients after feeding and mobilize them during a period of negative energy balance (Zuidhof, 2018). Deposition and mobilization of nutrients are not completely efficient processes (McCue 2006; de Beer and Coon, 2007) and probably repeated storage and mobilization of nutrients resulted in lower

efficiency of skip-a-day broiler breeders compared to daily fed broiler breeders (Zuidhof et al., 2015; de Beer and Coon, 2007).

2.4 Feed Efficiency and Energy Efficiency

2.4.1 Feed Efficiency

Feed conversion ratio is the most common measure of feed efficiency in the poultry industry. Feed conversion ratio is calculated as feed intake divided by BW gain. Moreover, FCR during the laying period is calculated as feed intake divided by egg mass. Feed conversion ratio can be calculated on a weekly basis and for overall performance of a flock. Although FCR is a good indicator of efficiency, it may not be the best one because it does not account for maintenance, BW gain, and egg production (where relevant) energy requirements at the same time. Decreased FCR does not necessarily mean an animal was biologically efficient because the FCR calculation did not adjust for feed intake, ME_m and production requirement at the same time (Koch et al., 1963). A broiler breeder with a low FCR may have a high ME_m and low production rate. 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 BW gain, ME_m and egg production.

2.4.2 Energy Efficiency

Residual feed intake (**RFI**) is a biological indicator of energetic efficiency and although it is more difficult to calculate, it provides better insight into the metabolic state of an animal. Residual feed intake is defined as the difference between observed and expected feed intake (Koch et al., 1963). The calculated average RFI in a population is zero with approximately half of the individuals above and half below the average. Romero et al. (2009); Pishnamazi et al. (2015); Zuidhof et al. (2014) also defined RFI as the difference between observed and expected energy

intake. Observed energy intake is calculated by multiplying the actual feed intake by the energy of a diet. However, predicted energy intake is estimated using an empirical energy intake model and accounts for energy used for maintenance (total HP), BW gain and production (Arthur and Herd, 2008). If ME intakes for animal A and B are equal and total HP by animal A is less than animal B it means that more energy is available for animal A to partition toward growth and production compared to animal B. Koch et al. (1963) and Crews (2005) reported that efficient beef cattle had lower RFI because they had lower feed intake without compromising the production level. Similarly, broiler chickens with low RFI had lower feed intake compared to broiler chickens with high RFI (Gabarrou et al., 1998). It was demonstrated that broiler chickens with low RFI had also lower ME_m relative to high RFI chickens (Geraert et al., 1991). Similarly, broiler chickens with low RFI had lower diet-induced thermogenesis and lost less energy as heat compared to broiler chickens with high RFI (Gabarrou et al. 1997; 1998).

On the other hand, RFI may not be the best indicator of the energetic efficiency of biological processes. 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 the total HP which results in a bias for increasing the RFI calculation. However, to understand which animal has released more total HP to the environment and was less efficient for the amount of ME intake that was consumed, the most reasonable comparison would occur when ME intake and total HP are estimated per unit of metabolic BW (kcal/kg). Residual maintenance ME (**RME_m**) is another energetic efficiency factor that can estimate efficiency without being confounded by feed intake (Romero et al., 2009). Residual maintenance ME is calculated by the residual of the linear relationship between total HP and ME intake. The slope of the linear equation between total HP and ME intake ($kcal/kg^b/kcal/kg^b$) defines the linear rate of

change of total HP with respect to ME intake (Romero et al., 2009). Since the units for the slope coefficient cancel, the slope can directly interpret as the proportion of dietary energy lose by pullets as heat. It defines the linear rate of change of total heat production with respect to ME intake. Hens with greater RME_m efficiency (lower ME_m) partitioned more energy toward chick production than hens with low RME_m efficiency (higher ME_m; Romero et al., 2009).

2.5 Energy, Different Energy Systems and Energy Requirement in Poultry

2.5.1 Utilization of Dietary Energy

Energy is not a nutrient per se. Oxidation of lipids (fats and oils), carbohydrates (starch, sugar, and fiber), and protein (amino acids) release energy in the body. Common systems to describe dietary energy are gross energy, digestible energy, metabolizable energy, and net energy. Gross energy (**GE**), or heat of combustion, is released after complete combustion of a compound with oxygen in a bomb calorimeter (Cocjin, 1990). Gross energy does not take into account any of the losses of energy during ingestion, digestion, and metabolism of feed. Digestible energy (**DE**) is the gross energy of feed minus the gross energy of feces but it does not take into account losses of energy in urine and gases during feed metabolism (Noblet, 2013). Metabolizable energy (**ME**) is defined as the digestible energy minus energy in urine and gases. Most of the energy lost in gases is due to methane production, which is negligible in poultry (Noblet, 2013). In poultry, excreta correspond to the combination of feces and urines thus ME takes into account losses of energy in poultry excreta (Noblet, 2013). Net energy (**NE**) is defined as metabolizable energy minus heat increment, which is the heat produced during digestion of feed, metabolism of nutrients and excretion of waste (Noblet, 2013). However, NE is much more difficult and more complex to determine than DE or ME because it is difficult to account for all levels of heat production, and this is the reason why NE is not used in poultry (Noblet, 2013). The ME values of feeds for poultry

can be easily found from feeding tables (Noblet, 2013). Apparent ME (**AME**) is much easier to measure than NE, moreover, the closest estimate to the NE of feed is the ME values and the ME accounts for the loses in excreta, thus the ME values of feed are reliable to use in poultry.

Metabolizable energy (**ME**) intake is partitioned to maintenance, growth, and egg production in broiler breeders. The ME intake is partitioned to maintenance, and growth during rearing period of broiler breeders. Metabolizable energy intake models are used to predict the energy partitioned to maintenance, growth, and egg production. Coefficients in the predicted ME intake models are used to describe ME requirements for ME_m, and growth and egg production of broiler breeders. The ME_m includes energy required for basal metabolism, level of activity, and thermal regulation (NRC, 1981; Emmans, 1994; Zuidhof, 2019) and since this energy is lost as heat, ME_m is equal to total HP (Zuidhof, 2019). Caged broiler breeders had 20% lower ME_m (total HP) compared to free-run broiler breeders because birds in cages have a lower activity level (Sakomura, 2004). Environmental temperature can also affect ME_m (total HP) by changing the rate of heat loss to the environment (Pishnamazi et al., 2015). Total HP decreased with increasing temperature to 24.3°C, after which total HP increased (Pishnamazi et al., 2015). Moreover, body composition affects total HP. Body protein had higher ME_m (total HP) than body fat (Close, 1990). Previous researchers have used modeling approaches to estimate ME requirements for maintenance, growth and egg production in broiler breeders (Reyes et al., 2011; Sakomura et al., 2003; Rabello et al., 2006; Romero et al., 2009; Pishnamazi et al., 2015).

2.5.2 Energy Partitioning

Broiler breeders partition ME toward maintenance, growth and egg production however during rearing period ME partitions only toward maintenance and growth. To quantify ME intake to maintenance, growth and egg production, empirical ME intake models are estimated

(Pishnamazi et al., 2015; Reyes et al., 2011; Sakomura et al., 2003; Zuidhof et al, 2014). Zuidhof et al. (2014) suggested the following non-linear mixed model for determining ME requirements for maintenance and growth in broilers:

$$\text{Observed MEI} = \text{Expected MEI} + \varepsilon$$

$$\text{Expected MEI} = (a + u) \times BW^{b} + c \times ADG$$

$$\text{Observed MEI} = (a + u) \times BW^{b} + c \times ADG + \varepsilon$$

where MEI was ME intake (kcal/d); a was ME_m or the average total HP for all pens (birds) for the entire experimental period (kcal/kg^b); $u \sim N(0,\sigma_u^2$; kcal/kg^b) was the random pen deviation from the average maintenance requirement such that $a + u_i$ was the estimated ME_m for each experimental pen; BW was the average BW (kg) of each experimental unit (pen) during each week, which was used to estimate total HP; BW^b was metabolic BW; c (kcal/g) was the coefficient of average daily gain (**ADG**, g/d) that defined the ME cost for each g of BW gain; and ε was the residual or unexplained error (RFI; kcal/d). Zuidhof et al. (2014) reported that coefficients for the nonlinear mixed model for MEI partitioning model produced the following equation:

$$\text{Observed MEI} = (195.96 + u) \times BW^{0.7543} + 2.4833 \times ADG + \varepsilon$$

The coefficient a = 195.96 predicted that broiler chickens required 195.96 kcal/kg$^{0.7543}$ for maintenance. The coefficient c = 2.4833 predicted that broilers needed 2.4833 kcal/g for BW gain.

Romero et al. (2009) suggested a linear model to partition ME intake to maintenance, growth, and egg mass in broiler breeders from 20 to 60 wk of age:

$$\text{Observed MEI} = 111.95\ BW^{0.75} - 0.36\ T \times BW^{0.75} + 3.36\ ADG + 2.10\ EM + \varepsilon$$

Where MEI (kcal/d) was ME daily intake, BW (kg); $BW^{0.75}$ was metabolic BW, T (°C) was temperature; ADG (g/d) was average daily gain; EM (g/d) was egg mass and ε was residual error. Thus the above ME intake model showed that broiler breeder hens required 111.95 kcal/$BW^{0.75}$ of

ME for maintenance (Total HP). The negative coefficient (-0.36) for T × $BW^{0.75}$ showed that the ME_m would be decreased with increasing temperatures to 21°C because the temperature was set at 21°C from 20 to 60 wk of age. Moreover, broiler breeder hens needed 3.36 kcal of ME for each g of gain and they required 2.10 kcal of ME for each g of egg mass.

Taken all together, both of these empirical modeling approaches (linear and non-linear mixed) can be used for energy partitioning in broiler breeders. However, the data model must converge to a solution, and estimated coefficients should have biological meaning. Even if the data model converges to a solution but the estimated coefficients do not have biological meaning, another modeling approach should be taken. Moreover, if the sample size is small usually the data model is converged but the estimated coefficients are probably not biologically feasible. Thus, it is important to have a sufficiently large sample size to estimate an empirical model that converges, and coefficients have biological meaning.

2.6 Endocrinology and Reproduction

2.6.1 Regulation of Feed Intake by the Central Nervous System

In poultry species, adjustments to feed intake and energy expenditure result in maintaining BW throughout their lifecycle (Richards and Proszkowiec-Weglarz, 2007). Researchers have assessed regulation of feed intake by the central nervous system (**CNS**) and peripheral tissue mechanisms in poultry (Richards, 2003; Kuenzel et al., 1999; Denbow, 1994). However, our understanding of the molecular mechanisms that control energy expenditure in poultry remains quite limited. The hypothalamic melanocortin system in the CNS comprises two types of neuropeptides to regulate feed intake; neuropeptide Y (**NPY**) and pro-opiomelanocortin (**POMC)** (Richards et al., 2010; Liu et al., 2016; Rubinstein and Low, 2017). Feed restriction reduced hypothalamic POMC mRNA levels in mice (Mizuno et al., 1998), but increased it in overfed rats

(Hagan et al., 1999). In contrast, feed restriction increased NPY mRNA levels in broiler chickens compared to *ad libitum* controls (Boswell et al., 1999). Stimulation of NPY-expressing (anabolic) neurons results in increasing feed intake and energy storage whereas stimulation of POMC-expressing (catabolic) neurons results in decreasing energy intake and storage (Richards et al., 2010). In response to lowered energy status, AMP-activated protein kinase (**AMPK**) is activated in the hypothalamus, which stimulates activity of NPY-expressing neurons and results in increased feed intake and reduced energy expenditure (Richards et al., 2010). Conversely, activation of mammalian target of rapamycin (**mTOR**), increased activity of the POMC-expressing (catabolic) neurons, which resulted in reducing feed intake and increasing energy expenditure (Wullschleger et al., 2006; Cota et al., 2006; Richards and Proszkowiec-Weglarz, 2007). The TOR gene homolog, located on chromosome 21, is expressed in chicken tissues including the hypothalamus (Richards and Proszkowiec-Weglarz, 2007), although the existence and functioning of a hypothalamic TOR signaling pathway in birds has not yet been demonstrated.

Other genes also have an important role in feed regulation (Liu et al., 2015). For example, leptin is the product of the obese gene and is released from adipose tissue (Friedman and Halaas, 1998). In birds, the leptin gene was eventually discovered confirming its potential physiological relevance (Seroussi et al., 2016; Friedman-Einat et al., 2014; Prokop et al., 2014; Wang et al., 2016). The leptin gene and the leptin receptor (**LEPR**) gene have been clearly identified for chickens and turkeys (Seroussi et al., 2016; Liu et al., 2007; Richards and Poch, 2003; Horev et al., 2000). Intracerebroventricular (**ICV**) injection of leptin suppressed feed intake in broiler chickens and in leghorns (Denbow et al., 2000). Leptin regulates food intake and energy homeostasis via its interaction with the LEPR in the hypothalamus (Friedman, 2011; Adachi et al., 2008). Leptin receptor is also co-expressed with NPY and POMC in the arcuate nucleus of the

hypothalamus (Silva et al., 2016; Elias et al., 1999). Interestingly, mRNA levels of hypothalamic neuropeptides, including NPY, POMC, and LEPR did not differ between broiler chickens and layers (Yuan et al., 2009). Therefore, expression of hypothalamic genes was very similar in different strains of birds. In broiler chickens, concentration of NPY increased with age from 0 to 7 d, whereas POMC and LEPR gene expression in hypothalamus gradually decreased with age from 0 to 7 d (Huang et al., 2010). This showed that POMC and NPY had reverse responses with increasing energy intake in broiler chickens, which was probably related to their functions in energy homeostasis. Increasing energy expenditure by increasing POMC gene expression and decreasing energy expenditure by increasing NPY gene expression work together to regulate energy balance (Richards et al., 2010). Thereby POMC and NPY genes promote the utilization of energy for maintenance, growth, and reproduction (Richards et al., 2010). In total, the balance in the activity of hypothalamic melanocortin system neurons ultimately determines whole body energy balance and BW (Richards et al., 2010; Richards and Proszkowiec-Weglarz, 2007). In response to lowered energy status due to feed restriction in broiler breeders, it is expected that activation of AMPK will stimulate feed intake through increasing the expression of NPY gene. Consequently, it is expected that a reduction in expression of the POMC gene will prevent a decrease in energy intake to maintain energy homeostasis. Moreover, the leptin gene, along with its receptor, suppresses feed intake, thus feed restriction is expected to result in an increase in leptin and leptin receptor gene expressions.

2.6.2 Neuroendocrine Control of Reproduction

The hypothalamic–pituitary–gonadal (**HPG**) axis controls reproduction in birds with each component secreting specific neuropeptides or hormones (Bédécarrats et al., 2009). In birds, reproduction is tightly regulated by stimulatory (gonadotropin releasing hormone; **GnRH**) and

inhibitory (gonadotropin inhibitory hormone; **GnIH**) hypothalamic neuropeptides (Kriegsfeld et al., 2015; Tsutsui et al., 2012; Bédécarrats et al., 2016; Bédécarrats 2015; Baxter et al., 2014; Bédécarrats et al, 2009). During the scotophase (predominant under short days), melatonin is produced by the retina of the eye and the pineal gland stimulates the synthesis and release of GnIH resulting in the tonic inhibition of the reproductive axis (Bédécarrats et al., 2016). During the photophase (predominant under long days), a reduction in melatonin and stimulation of hypothalamic photoreceptors trigger the release of GnRH-I in the median eminence (Bédécarrats et al., 2016). Therefore, GnRH is released by the hypothalamus and binds to its receptors in the anterior pituitary gland (Bédécarrats et al., 2009). In chickens, two specific G-coupled protein receptors have been identified for GnRH-I in the anterior pituitary gland, **GnRH-RI** and **GnRH-RII** (Sun et al., 2001; Shimizu and Bédécarrats, 2006). The GnRH-RII is a type III receptor, thus GnRH-RII was renamed to **GnRH-RIII** (Joseph et al., 2009). GnRH-RI is expressed in the brain, pituitary, and gonads (Sun et al., 2001). However, GnRH-RIII is pituitary-specific (Shimizu and Bédécarrats, 2006). Both GnRH-RI and GnRH-RIII are G-protein coupled receptors and were shown to couple not only to the Gq alpha subunit, resulting an increase in intracellular inositol phosphates (Sun et al*.,* 2001; Shimizu and Bédécarrats, 2006), but also couples to the Gs alpha subunit, causing an activation of cyclic adenosine monophosphate (**cAMP**) pathway (Shimizu and Bédécarrats, 2010). Inositol phosphates stimulate the release of Ca^{2+} from an extracellular space to intracellular space in combination with diacylglycerol. Moreover, an increase in concentration of cAMP leads to the activation of protein kinase A (**PKA**) and once protein kinase A is activated, it phosphorylates a number of proteins including transcription factors, which regulate gene expression (Bédécarrats et al., 2009). Increased intracellular Ca^{2+} stimulates the release of gonadotropins from the anterior pituitary (Bédécarrats et al., 2009). Gonadotropin releasing

hormone stimulates the release of follicle stimulating hormone (**FSH)**, and luteinizing hormone, (**LH**) from the anterior pituitary gland (Bédécarrats et al., 2009). Gonadotropins (LH, and FSH) trigger gonadal development and the synthesis of steroid hormones such as estradiol (**E2**) from the small follicles of the ovary and progesterone from the granulosa cells of the large follicles (Robinson and Etches, 1986; Bédécarrats et al., 2009; Tsutsui et al., 2010; Dunn et al., 2009).

Hypothalamic 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). Gonadotropin inhibitory hormone is released into the portal vascular system to reach the anterior pituitary (Tsutsui et al., 2000). In chickens, GnIH receptor (**GnIH-R**) is expressed in the pituitary gland on both LH and FSH producing cells (Maddineni et al., 2008). Similarly, to GnRH receptors, GnIH-R is a G-protein coupled receptor, and it couples to the Gi alpha subunit, which inhibits the activity of adenylyl cyclase and the production of cAMP. Therefore, GnIH and its receptor maintain a hen in a juvenile state (Shimizu and Bédécarrats, 2010; Bédécarrats et al., 2009). As maturation progresses, GnRH release dominates GnIH, and in the pituitary, the ratio of GnIH-R to GnRH-RIII switches toward GnRH-RIII and the HPG axis becomes fully functional (Bédécarrats et al., 2009). In mature hens, high levels of E2 and progesterone maintain inhibition of the GnIH-R, moreover, E2 may also maintain high levels of GnRH-RIII (Bédécarrats et al., 2009).

2.6.3 Age, Body Weight, and Body Composition at Sexual Maturation

Sexual maturation is defined as the age at first oviposition (onset of lay; Robinson et al., 1998; Pishnamazi et al., 2014). Photostimulation (van der Klein et al., 2018b), BW (van der Klein et al., 2018a), age (HPG axis maturation; Bédécarrats et al., 2016), and body composition (Nonis and Gous, 2016; Renema et al., 1999) affect the onset of sexual maturation. Broiler breeder hens (van der Klein et al., 2018a) and laying hens (Summers and Leeson, 1983) with greater BW and

greater lipid content entered into lay earlier compared to birds with lower BW and lower lipid content. Similarly, Renema et al. (1999) reported that after photostimulation, onset of lay was advanced for pullets fed *ad libitum* compared to feed restricted pullets. van der Klein et al. (2018a) indicated that a fat mass threshold may be required for the onset of lay and these authors showed that at 55 wk of age, the proportion of breast muscle did not differ between breeders fed to meet the standard breeder-recommended target BW and a high BW treatment group reaching the standard 21 wk BW at 18 wk. Zuidhof et al. (2014) reported that body composition in broiler chickens changed from 1957 to 2005. They demonstrated that broilers in 2005 had less body fat and more body protein compared to broiler chickens in 1957 and 1978. This indicates that the body composition of modern broiler breeders, the parents of broiler chickens, have likely changed as well. These changes in body composition of broiler breeders could be due to severe feed restriction. Feed restriction for modern broiler breeders has become more severe (van Emous, 2015) due to increased growth potential of broiler breeders during the last 30 years (Renema et al., 2007). van der Klein et al. (2018a) showed that hens on a standard BW treatment had lower proportional fat pad weight (1.6%) at 55 wk of age compared to the hens on a high BW treatment reaching the standard 21 wk BW at 18 wk (2.2%). Moreover, broiler breeders fed according to the breeder-recommended target BW curve had lower egg production compared to broiler breeders fed on a 22% heavier target BW curve reaching the standard 21 wk BW at 18 wk (129.4 vs. 92.8, respectively; van der Klein et al., 2018). These authors suggested that the current recommended breeder BW is probably too low for optimal sexual maturation of hens. Broiler breeders may require relaxation in the degree of feed restriction to help them to increase their energy intake and deposit more energy as fat and have more energy available to partition toward egg production at the onset of sexual maturation and during the entire laying period.

2.7 Conclusion

Among different feed restriction methods in broiler breeders, the limited daily feed restriction increased feed efficiency through reduced storage and mobilization of nutrients. On the other hand, broiler breeder hens fed on a 5-2 restricted feeding program during rearing period had larger eggs during laying period when switched to daily restricted feeding compared to hens fed on daily restricted feeding during rearing and laying period due to increased lipogenesis. However, there is currently lack of a commercial feed restriction method that increases feed efficiency through reduced storage and mobilization of nutrients and also results in producing large eggs in the same time. Moreover, nutrient availability from the gut fluctuates in daily, skip-a-day, and 5-2 restricted feeding. It seems that a new feeding system needs to be developed to increase efficiency through reduced storage and mobilization of nutrients while it provides triggers (such as adequate fat content) for the onset of sexual maturation. Recently, a new feeding system named precision feeding (**PF**) system was developed at the University of Alberta to allocate feed to individual birds to reach specific target BW. The PF system provides several small meals per day for each individual bird; thus it reduces cyclic deposition and mobilization of nutrients and it increases the efficiency due to consistency in the supply of nutrients. Moreover, the PF system increased fertility compared to a daily restricted feeding system. In addition to efficiency and fertility, other factors are important in the management of broiler breeders. For example, body composition, energy intake levels, photostimulation, HPG axis maturation (age of bird), and BW are triggers for the onset of sexual maturation in broiler breeders. However, our understanding of the molecular mechanisms in poultry, especially under different energy intake levels, is quite limited, and we need to evaluate the effect of different energy intake levels on the molecular mechanisms of gene expression to achieve energy balance. Thus, the impact of energy intake levels

on HPG axis maturation, body composition, onset of sexual maturation, energy homeostasis needs to be further studied. Research is required to determine whether the frequency of feeding can increase feed and energetic efficiency during the rearing and laying periods in broiler breeders. Moreover, research is required to assess the effect of different energy intake levels on the onset of sexual maturation to further enhance reproduction in broiler breeders. Additionally, research is needed to evaluate the effect of different energy intake levels on the molecular mechanism of gene expression to achieve energy balance.

2.8 Objectives

1) The first objective was to develop a ME intake model whose coefficients describe ME cost for total HP and growth in broiler breeder pullets (Chapter 3).

2) The second objective was to evaluate energy efficiency using RFI and residual heat production and feed efficiency using FCR in broiler breeder pullets fed using PF and skip-a-day feeding systems (Chapter 3).

3) The third objective was to develop a ME intake model whose coefficients quantify the amount of ME partitioned to total HP, growth, and egg mass of broiler breeder hens (Chapter 4).

4) The fourth objective was to compare the energetic efficiency using RFI between conventional daily restricted feeding program and the PF system in broiler breeder hens (Chapter 4).

5) The fifth objective was to evaluate the effect of a conventional daily restricted feeding program and the PF system on carcass composition and age at 50% production (Chapter 4).

6) The sixth objective was to assess the effect of ME intake on sexual maturation, specifically on mRNA levels of GnRH-I and GnIH in the hypothalamus and their receptors in the anterior pituitary gland in broiler breeder pullets (Chapter 5).

7) The seventh objective was to investigate the effect of ME intake on plasma concentration of reproductive hormones (LH, FSH, and E2) in broiler breeder pullets (Chapter 5).

8) The eighth objective was to evaluate the effect of ME intake on the expression of POMC, NPY and LEPR genes to understand how energy balance is regulated by the hypothalamus in broiler breeder pullets at post-photostimulation and during sexual maturity (Chapter 5).

9) The ninth objective was to investigate the effect of ME intake on carcass composition in broiler breeder pullets (Chapter 5).

2.9 Hypotheses

It was hypothesized that:

1) Precision-fed broiler breeder pullets would be more efficient compared to pullets fed using conventional skip-a-day feeding method because of a higher feeding frequency (Chapter 3).

2) Precision-fed broiler breeder hens would be more energetically efficient because of reduced storage and mobilization of nutrients by providing several small meals in a day and would partition more energy toward egg production and increase egg production compared to the hens in the conventional daily restricted feeding program (Chapter 4).

3) Higher ME intake of broiler breeder pullets would increase GnRH-I mRNA levels in the hypothalamus and also increase the mRNA levels of GnRH receptors in the pituitary to advance the onset of lay (Chapter 5).

4) Higher ME intake of broiler breeder pullets would reduce GnIH mRNA levels in the hypothalamus and suppress the mRNA levels of GnIH-R in the pituitary at the onset of lay (Chapter 5).

5) An advance in sexual maturation driven by higher mRNA levels of GnRH-I and lower expression of GnIH gene would translate into an increase in the concentrations of reproductive hormones after photostimulation (Chapter 5).

6) Higher ME intake in broiler breeder pullets would decrease the expression of NPY gene and increase the expression of POMC and LEPR genes to maintain energy homeostasis (Chapter 5).

7) Higher ME intake would increase carcass lipid and decrease carcass protein in broiler breeder pullets at the onset of lay (Chapter 5).

2.10 References

Adachi, H., Y. Takemoto, T. Bungo, and T. Ohkubo. 2008. Chicken leptin receptor is functional in activating JAK-STAT pathway in vitro. J. Endocrinol. 197:335–342.

Arthur, J. P. F., and R. M. Herd. 2008. Residual feed intake in beef cattle. R. Bras. Zootec. 37:269–279.

Aviagen, 2018. Ross 308 parent stock management handbook. Aviagen Inc., Huntsville, AL. Accessed Feb 2019. http://en.aviagen.com/assets/Tech_Center/BB_Resources_Tools/Pocket_Guides/Ross-PS-Pocket-Guide2013EN.pdf.

Baxter, M., N. Joseph, V. R. Osborne, and G. Y. Bedecarrats. 2014. Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. Poult. Sci. 93:1289–1297.

Becker, W. A., J. V. Spencer, L. W. Mirosh, and J. A. Verstrate. 1979. Prediction of fat and fat free live weight in broiler chickens using back skin fat, abdominal fat and live body weight. Poult. Sci. 58:835–842.

Bédécarrats, G. Y. 2015. Control of the reproductive axis: Balancing act between stimulatory and inhibitory inputs. Poult. Sci. 94:810–815.

Bédécarrats, G. Y., M. Baxter, and B. Sparling. 2016. An updated model to describe the neuroendocrine control of reproduction in chickens. Gen. Comp. Endocrinol. 227:58–63.

Bédécarrats, G. Y., H. McFarlane, S. R. Maddineni, and R. Ramachandran. 2009. Gonadotropin-inhibitory hormone receptor signaling and its impact on reproduction in chickens. Gen. Comp. Endocrinol. 163:7–11.

Blem, C. R. 1990. Current ornithology. Pages 59-113 in Avian Energy Storage. D. M. Power, ed. Plenum Press, New York, United States.

Boswell, T., I. C. Dunn, and S. A. Corr. 1999. Hypothalamic neuropeptide Y mRNA is increased after feed restriction in growing broilers. Poult. Sci. 78:1203–1207.

Bowmaker, J. E., and R. M. Gous. 1989. Quantification of reproductive changes and nutrient requirements of broiler breeder pullets at sexual maturity. Br. Poult. Sci. 30:663–675.

Bruggeman, V., O. Onagbesan, E. D'Hondt, N. Buys, M. Safi, D. Vanmontfort, L. Berghan, F. Vandesande, E. Decuypere. 1999. Effects of timing and duration of feed restriction during rearing on reproductive characteristics in broiler breeder females. Poult. Sci. 78:1424–1434.

Close, W. H. 1990. The evaluation of feeds through calorimetry studies. Pages 21–39 in Feed Stuff Evaluation. Butterworths. J. Wiseman D. J. A. Cole, ed. The University Press, Cambridge, United Kingdom.

Cobb-Vanress. 2005. Cobb 500 Breeder Management Guide: Blueprint for Success. Cobb-Vantress, Siloam Springs, AR.

Cobb Vantress. 2008. Cobb Broiler Management Guide: Blueprint for Success. Cobb-Vantress, Siloam Springs, AR.

Cocjin, B. B. 1990. Terms commonly used in feeding and nutrition. Pages 135–138 in Principles of Animal Science. Rex Book Store, Manila, Philippines.

Cota, D., K. Proulx, K. A. Smith, S. C. Kozma, G. Thomas, S. C. Woods, and R. J. Seeley. 2006. Hypothalamic mTOR signaling regulates food intake. Science. 312:927–930.

Crews, D. H. D. 2005. Genetics of efficient feed utilization and national cattle evaluation: A review. Genet. Mol. Res. 4:152–165.

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927–1939.

de Beer, M., R. W. Rosebrough, B. A. Russell, S. M. Poch, M. P. Richards, and C. N. Coon. 2007. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. Poult. Sci. 86:1726–1738.

de Jong, I. C., D. Guemene. 2011. Major welfare issues in broiler breeders. World. Poult. Sci. 67:73–81.

de Jong, I. C., B. Jones. 2006. Feed restriction and welfare in domestic birds. Pages 120-135 in Feeding in Domestic Vertebrates. V. Bels, ed. CABI, Wallingford, United Kingdom.

Denbow, D. M. 1994. Peripheral regulation of food intake in poultry. J. Nutr. 124:1349S–1354S.

Denbow, D. M., S. Meade, A. Robertson, J. P. McMurtry, M. Richards, and C. Ashwell. 2000. Leptin-induced decrease in food intake in chickens. Physiol. Behav. 69:359–362.

Dunn, I. C., N. A. Ciccone, and N. T. Joseph. 2009. Endocrinology and genetics of the hypothalamic-pituitary-gonadal axis. Pages 61–88 in Biology of Breeding Poultry. P. M. Hocking, ed. CABI, Wallingford, Oxfordshire, United Kingdom.

Emmans, G. C. 1994. Effective energy: a concept of energy utilization applied across species. Br. J. Nutr. 71:801–821.

Fattori, T. R., H. R. Wilson, R. H. Harms, F. B. Mather, R. D. Miles, and G. D. Butcher. 1993. Response of broiler breeder females to feed restriction below recommended levels. 3. Characterizing the onset of sexual maturity. Poult. Sci. 72:2044–2051.

Friedman, J. M. 2011. Leptin and the regulation of body weight. Keio. J. Med. 60:1–9.

Friedman-Einat M., L. A. Cogburn, S. Yosefi, G. Hen, D. Shinder, A. Shirak, and et al. 2014. Discovery and characterization of the first genuine avian leptin gene in the rock dove (Columba livia). Endocrinol. 155:3376–84.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. Br. Poult. Sci.39:79–89.

Gabarrou, J. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. J. Nutr. 127:2371–2376.

Geraert, P. A., S. Guillaumin, A. Bordas and P. Merat. 1991. Evidence of a genetic control of diet-induced thermogenesis in poultry. Proc. 12th Symp. Eur. Anim. Prod. 58:380–383.

Gilmet, T.E.R.M. 2015. Behavioural implications of precision feeding broiler breeders. MSc Diss. Univ. Alberta, Edmonton, Alberta.

Goerzen, P. R., and W. L. Julsrud, and F. E. Robinson. 1996. Duration of fertility in ad libitum and feed-restricted caged broiler breeders. Poult. Sci. 75:962–965.

Guglielmo, C. G. 2018. Obese super athletes: fat-fueled migration in birds and bats. J. Exp. Biol. 221:pmid:29514885.

Hadinia, S. H., P. R. O. Carneiro, C. A. Ouellette, and M. J. Zuidhof. 2018. Metabolizable energy partitioning of broiler breeder pullets in skip-a-day and precision feeding systems. Poult. Sci. 97:4279–4289.

Hadinia, S. H., P. R. O. Carneiro, D. R. Korver, and M. J. Zuidhof. 2019. Energy partitioning by broiler breeder hens in conventional daily-restricted feeding and precision feeding systems. Poult. Sci. 0:1–12. http://dx.doi.org/10.3382/ps/pez387.

Hagan, M., P. A. Rushing, M. W. Schwartz, K. A. Yagaloff, P. Burn, S. C. Woods, R. J. Seeley. 1999. Role of the CNS melanocortin system in response to overfeeding. J. Neurosci. 19:2362–2367.

Heck, A., O. Onagbesan, K. Tona, S. Metayer, J. Putterflam, Y. Jego, J. J. Trevidy, E. Decuypere, J. Williams, M. Picard and V. Bruggeman. 2004. Effects of ad libitum feeding on performance of different strains of broiler breeders. Br. Poult. Sci. 45:695–703.

Hocking, P. M. 2006. High-fibre pelleted rations decrease water intake but do not improve physiological indexes of welfare in food-restricted female broiler breeders. Br. Poult. Sci. 47:19–23.

Hocking, P. M., R. Bernard, and G. W. Robertson. 2002. Effects of low dietary protein and different allocations of food during rearing and restricted feeding after peak rate of lay on egg production, fertility and hatchability in female broiler breeders. Br. Poult. Sci. 43:94–103.

Hocking, P. M., M. H. Maxwell, G. W. Robertson, and M. A. Mitchell. 2002. Welfare assessment of broiler breeders that are food restricted after peak of lay. Br. Poult. Sci. 43:5–15.

Horev, G., P. Einat, T. Aharoni, Y. Eshdat, and M. Friedman-Einat. 2000. Molecular cloning and properties of the chicken leptin-receptor (CLEPR) gene. Mol. Cell. Endocrinol. 162:95–106.

Huang, J. X., L. Lu, L. Xi, X. G. Luo, and B. Liu. 2010. Effects of age and strain on the expression of leptin receptor, neuropeptide Y and pro-opiomelanocortin in the hypothalamus of young chickens. Br. Poult. Sci. 51:696–702.

Johnson, P. A. 2012. Follicle selection in the avian ovary. Reprod. Dom. Anim. 47:283–287.

Johnston, D. W. 1970. Caloric density of avian adipose tissue. Comp. Biochem. Physiol. 34: 827–832.

Jones, E. K. M., V. Zaczek, M. MacLeod, and P. M. Hocking. 2004. Genotype, dietary manipulation and food allocation affect indices of welfare in broiler breeders. Br. Poult. Sci. 45:725–37.

Joseph, N. T., K. Morgan, R. Sellar, D. McBride, R. P. Millar, and I. C. Dunn. 2009. The chicken type III GnRH receptor homologue is predominantly expressed in the pituitary, and exhibits similar ligand selectivity to the type I receptor. J. Endocrinol. 202:179–190.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22:486–494.

Kriegsfeld, L. J., T. Ubukac, G. E. Bentley, and K. Tsutsui. 2015. Seasonal control of gonadotropin-inhibitory hormone (GnIH) in birds and mammals. Front. Neuroendocrinol. 37:65–75.

Kuenzel, W. J., M. M. Beck, and R. Teruyama. 1999. Neural sites and pathways regulating food intake in birds: A comparative analysis to mammalian systems. J. Exp. Zool. 283:348–364.

Leeson, S., and J. D. Summers. 2001. Energy. Pages 34–99 in Nutrition of the Chicken. 4th ed. Univ. Books, Guelph, ON, Canada.

Leeson, S., and J. D. Summers. 1991. Feeding programs for broiler breeders. Pages 297–342 in Commercial Poultry Nutrition. 3rd ed. Nottingham. Univ. Press, Guelph, ON, Canada.

Liu, X., I. C. Dunn, P. J. Sharp, and T. Boswell. 2007. Molecular cloning and tissue distribution of a short form chicken leptin receptor mRNA. Domest. Anim. Endocrinol. 32:155–166.

Liu, L., S. Xu, X. Wang, H. Jiao, J. Zhao, and H. Lin. 2016. Effect of dexamethasone on hypothalamic expression of appetite-related genes in chickens under different diet and feeding conditions. J. Anim. Sci. Biotechnol. 7:23.

Maddineni, S., O. M. Ocon-Grove, S. M. Krzysik-Walker, G. L. Hendricks, J. A. Proudman, and R. Ramachandran. 2008. Gonadotrophin-inhibitory hormone receptor expression in the chicken pituitary gland: Potential influence of sexual maturation and ovarian steroids. J. Neuroendocrinol. 20:1078–1088.

McCue, M. D. 2006. Specific dynamic action: a century of investigation. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 144A:381–394.

Mench, J. A. 2002. Broiler breeders: Feed restriction and welfare. World's Poult. Sci. J. 58:23–29.

Merlet, F., J. Puterflam, J. M. Faure, P. M. Hocking, M. S. Magnusson, and M. Picard. 2005. Detection and comparison of time patterns of behaviours of two broiler breeder genotypes fed ad libitum and two levels of feed restriction. Appl. Anim. Behav. Sci. 94:255–271.

Mizuno, T. M., S. P. Kleopoulos, H. T. Bergen, J. L. Roberts, C. A. Priest, C. V. Mobbs. 1998. Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. Diabetes. 47:294–297.

Mohiti-Asli, M., M. Shivazad, M. Zaghari, M. Rezaian, S. Aminzadeh, and G. G. Mateos. 2012. Effects of feeding regimen, fiber inclusion, and crude protein content of the diet on performance and egg quality and hatchability of eggs of broiler breeder hens. Poult. Sci. 91:3097–3106.

Morrissey, K. L. H., T. Widowski, S. Leeson, V. Sandilands, A. Arnone, and S. Torrey. 2014. The effect of dietary alterations during rearing on growth, productivity, and behavior in broiler breeder females. Poult. Sci. 93:285–295.

National Research Council. 1981. Nutritional Energetics of Domestic Animals and Glossary of Energy Terms. 2nd ed. Natl. Acad. Press, Washington, DC, United States.

Nelson, D. L., and M. M. Cox. 2006. Bioenergetics and metabolism. Pages 481–488 in Lehninger Principle of Biochemistry. 4th ed. W. H. Freeman, Wisconsin, MDN, United States.

Noblet, J. 2013. Energy evaluation of pigs and poultry feeds: history, recent advances and applications to tropical conditions. 26th Annual PHILSAN Convention**,** Yapak, Boracay, Philippines.

Nonis, M. K., and R. M. Gous. 2016. Changes in the feather-free body of broiler breeder hens after sexual maturity. Anim. Product. Sci. 56:1099–1104.

Onagbesan, O. M., S. Metayer, K. Tona, J. Williams, E. Decuypere, and V. Bruggeman. 2006. Effects of genotype and feed allowance on plasma luteinizing hormones, follicle-stimulating hormones, progesterone, estradiol levels, follicle differentiation, and egg production rates of broiler breeder hens. Poult. Sci. 85:1245–1258.

Pishnamazi, A., R. A. Renema, D. C. Paul, I. I. Wenger, and M. J. Zuidhof. 2015. Effects of environmental temperature and dietary energy on energy partitioning coefficients of female broiler breeders. J. Anim. Sci. 93:4734–4741.

Pishnamazi, A., R. A. Renema, M. J. Zuidhof, and F. Robinson. 2014. Effect of age at photostimulation on sexual maturation in broiler breeder pullets. Poult. Sci. 93:1274–1281.

Pishnamazi, A., R. A. Renema, M. J. Zuidhof, and F. E. Robinson. 2008. Effect of initial full feeding of broiler breeder pullets on carcass development and body weight variation. Poult. Sci. 17:505–514.

Prokop, J. W., C. Schmidt, D. Gasper, R. J. Duff, A. Milsted, T. Ohkubo, and et al. 2014. Discovery of the elusive leptin in birds: identification of several "missing links" in the evolution of leptin and its receptor. PLoS. One. 9:e92751.

Pullar, J. D., and A. J. F. Webster. 1977. The energy cost of fat and protein deposition in the rat. Br. J. Nutr. 37:355–361.

Rabello, C. B. V., N. K. Sakomura, F. A. Longo, H. P. Couto, C. R. Pacheco, and J. B. K. Fernandes. 2006. Modelling energy utilisation in broiler breeder hens. Br. Poult. Sci. 47:622–631.

Renema, R. A., and F. E. Robinson. 2004. Defining normal: comparison of feed restriction and full feeding of female broiler breeders. World's Poult. Sci. 60:508–522.

Renema, R. A., F. E. Robinson, and P. R. Goerzen. 2001. Effects of altering growth curve and age at photostimulation in female broiler breeders. 1. Reproductive development. Can. J. Anim. Sci. 81:467–476.

Renema, R. A., F. E. Robinson, M. Newcombe, and R. I. Mckay. 1999. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 1. Growth and carcass characteristics. Poult. Sci. 78:619–628.

Renema, R. A., M. E. Rustad, and F. E. Robinson. 2007. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. World's Poult. Sci. J. 63:457–472.

Reyes, M. E., C. Salas, and C. N. Coon. 2011. Energy requirement for maintenance and egg production for broiler breeder hens. Int. J. Poult. Sci. 10:913–920.

Richards, M. P. 2003. Genetic regulation of feed intake and energy balance. Poult. Sci. 82:907–916.

Richards, M. P., and M. Proszkowiec-Weglarz. 2007. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poult. Sci. 86:1478–1490.

Richards, M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMurtry. 2010. Feed intake regulation for the female broiler breeder: In theory and in practice. J. Appl. Poult. Res. 19:182–193.

Robinson, F. E., and R. J. Etches. 1986. Ovarian steroidogenesis during follicular maturation in the domestic fowl (*Gallus domesticus*). Biol. Reprod. 35:1096–1105.

Robinson, F. E., R. A. Renema, L. Bouvier, J. J. R. Feddes, J. L. Wilson, M. Newcombe, and R. I. McKay. 1998. Effects of photostimulatory lighting and feed allocation in female broiler breeders. 1. Reproductive development. Can. J. Anim. Sci. 78:603–613.

Rodriguez, A. 2017. Effects of daytime and supplemental light spectrum on broiler breeder growth and sexual maturation. MSc Diss. Univ. Guelph, Guelph, Ontario.

Romero, L. F., M. J. Zuidhof, R. A. Renema, A. Naeima, and F. E. Robinson. 2009. Characterization of energetic efficiency in adult broiler breeder hens. Poult. Sci. 88:227–235.

Romero., L. F., J. S. Sands, S. E. Indrakumar, P. W. Plumstead, S. Dalsgaard, and V. Ravindran. 2014. Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease. Poult. Sci. 93:2501–2513.

Rubinstein, M., M. J. Low. 2017. Molecular and functional genetics of the proopiomelanocortin gene, food intake regulation and obesity. FEBS. Lett. 17:2593–2606.

Sakomura, N. K. 2004. Modeling energy utilization in broiler breeders, laying hens and broilers. Braz. J. Poult. Sci. 6:1–11.

Sakomura, N. K., R. Silva, H. P. Couto, C. Coon, and C. R. Pacheco. 2003. Modeling metabolizable energy utilization in broiler breeder pullets. Poult. Sci. 82:419–427.

Savory, C. J., A. Carlisle, M. H. Maxwell, M. A. Mitchell, and G. W., Robertson. 1993. Stress, arousal and opioid peptide-like immune reactivity in restricted-and ad lib fed broiler breeder fowls. Comp. Biochem. Physiol. 106A:587–594.

Savory, C. J., P. M. Hocking, J. S. Mann, and M. H. Maxwell. 1996. Is broiler breeder welfare improved by using qualitative rather than quantitative food restriction to limit growth rate? Anim. Welf. 5:105–127.

Schneider, B., M. Zuidhof, F. Robinson, and R. Renema. 2005. Allocating feed to female broiler breeders: Technical Bulletin #2. Accessed Jul. 2019. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/pou9452.

Seroussi, E., Y. Cinnamon, S. Yosefi, O. Genin, J. G. Smith, N. Rafati, S. Bornelöv, L. Andersson, and M. Friedman-Einat. 2016. Identification of the long-sought leptin in chicken and duck: expression pattern of the highly GC-rich avian leptin fits an autocrine/paracrine rather than endocrine function. Endocrinol. 157:737–751.

Shils, M. E., M. Shike, A. C. Ross, B. Caballero, and R. J. Cousins. 2006. Energy needs: assessment and requirements. Pages 136–138 in Modern Nutrition in Health and Disease. N. F. Butte, and B. Caballero, ed. Lippincott Williams and Wilkins, New York, United States.

Shimizu, M., and G. Y. Bédécarrats. 2010. Activation of the chicken gonadotropin-inhibitory hormone receptor reduces gonadotropin releasing hormone receptor signaling. Gen. Comp. Endocrinol. 167:331–337.

Shimizu, M., and G. Y. Bédécarrats. 2006. Identification of a novel pituitary-specific chicken gonadotropin-releasing hormone receptor and its splice variants. Biol. Reprod. 75:800–808.

Silva, J. P., G. Lambert, D. van Booven, and C. Wahlestedt. 2016. Epigenomic and metabolic responses of hypothalamic POMC neurons to gestational nicotine exposure in adult offspring. Genom. Med. 8:93.

Summers, J. D., and S. Leeson. 1983. Factors influencing early egg size. Poult. Sci. 62:1155–1159.

Sun, Y. M., I. C. Dunn, E. Baines, R. T. Talbot, N. Illing, R. P. Millar, and P. J. Sharp. 2001. Distribution and regulation by oestrogen of fully processed and variant transcripts of gonadotropin releasing hormone I and gonadotropin releasing hormone receptor mRNAs in the male chicken. J. Neuroendocrinol. 13:37–49.

Thomas, V. G., S. K. Mainguy, and J. P. Prevett. 1983. Predicting fat-content of geese from abdominal fat weight. J. Wildl. Manage. 47:1115–1119.

Tolkamp, B. J., and R. B. D'Eath. 2016. Hunger associated with restricted feeding systems. Pages 11-27 in Nutrition and the Welfare of Animals Farm. C. J. C. Phillips, ed. Springer, Cham, Switzerland.

Tsutsui, K., G. E. Bentley, G. Bedecarrats, T. Osugi, T. Ubuka, and L. J. Kriegsfeld. 2010. Gonadotropin-inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. Front. Neuroendocrinol. 31:284–295.

Tsutsui, K., E. Saigoh, K. Ukena, H. Teranishi, Y. Fujisawa, M. Kikuchi, S. Ishii, and P. J. Sharp. 2000. A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem. Biophys. Res. Commun. 275:661–667.

Tsutsui, K., T. Ubuka, G. E. Bentley, and L. J. Kriegsfeld. 2012. Gonadotropin-inhibitory hormone (GnIH): discovery, progress and prospect. Gen. Comp. Endocrinol. 177:305–314.

van der Klein, S. A. S., G. Y. Bédécarrats, F. E. Robinson, and M. J. Zuidhof. 2018a. Early photostimulation at the recommended body weight reduced broiler breeder performance. Poult. Sci. 97:3736–3745.

van der Klein, S. A. S., G. Y. Bédécarrats, and M. J. Zuidhof. 2018b. The effect of rearing photoperiod on broiler breeder reproductive performance depended on body weight. Poult. Sci. 97:3286–3294.

van Emous, R. 2015. Body composition and reproduction in broiler breeders: impact of feeding strategies. PhD Diss. Univ. Wageningen, Wageningen, the Netherland.

Wang, T., C. C. Hung, and D. J. Randall. 2006. The comparative physiology of food deprivation: from feast to famine. Annu. Rev. Physiol. 2006. 68:223–251.

Wang, D., C. Xu, T. Wang, H. Li, Y. Li, J. Ren, and et al. 2016. Discovery and functional characterization of leptin and its receptors in Japanese quail (Coturnix japonica). Gen. Comp. Endocrinol. 225:1–12.

Wei, Z., P. Li, S. Huang, P. Lkhagvagarav, M. Zhu, C. Liang, and C. Jia. 2019. Identification of key genes and molecular mechanisms associated with low egg production of broiler breeder hens in ad libitum. BMC. Genom. 20:408.

Williams, J. 2012. Ovarian dysfunction in broiler breeder hens. Medycyna. Wet. 68: 131–138.

Wullschleger, S., R. Loewith, and M. N. Hall. 2006. TOR signaling in growth and metabolism. Cell 124:471–484.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt. 1992. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. Poult. Sci. 71:1750–1761.

Yuan, L., Y. Ni, S. Barth, Y. Wang, R. Grossmann, and R. Zhao. 2009. Layer and broiler chicks exhibit similar hypothalamic expression of orexigenic neuropeptides but distinct expression of genes related to energy homeostasis and obesity. Brain. Res. 1273:18–28.

Yang, S., Y. Suh, Y. M. Choi, S. Shin, J. Y. Han, K. Lee. 2013. Loss of fat with increased adipose triglyceride lipase-mediated lipolysis in adipose tissue during laying stages in quail. Lipids. 48:13–21.

Zuidhof, M. J. 2018. Lifetime productivity of conventionally and precision-fed broiler breeders. Poult. Sci. 97:3921–3937.

Zuidhof, M. J. 2019. A review of dietary metabolizable and net energy: Uncoupling heat production and retained energy. J. Appl. Poult. Res. 28:231–241.

Zuidhof, M. J., M. V. Fedorak, C. C. Kirchen, E. H. M. Lou, C. A. Ouellette, and I. I. Wenger. 2016. System and method for feeding animals. Precision ZX, Inc., assignee. US Pat. Application No. 15/283,125.

Zuidhof, M. J., M. V. Fedorak, C. A. Ouellette, and I. I. Wenger. 2017. Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity. Poult. Sci. 96:2254–2263.

Zuidhof, M. J., D. E. Holm, R. A. Renema, M. A. Jalal, and F. E. Robinson. 2015. Effects of broiler breeder management on pullet body weight and carcass uniformity. Poult. Sci. 94:1389–1397.

Zuidhof, M. J., F. E. Robinson, J. J. R. Feddes, and R. T. Hardin. 1995. The effects of nutrient dilution on the well-being and performance of female broiler breeders. Poult. Sci. 74:441–456.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth efficiency, and yield of commercial broilers from 1957, 1978 and 2005. Poult. Sci. 93:2970–2982.

3 Chapter 3. Energy Partitioning by Broiler Breeder Pullets in Skip-a-day and Precision Feeding Systems

3.1 Abstract

An empirical nonlinear mixed model was derived to describe metabolizable energy (**ME**) partitioning in Ross 308 broiler breeder pullets. Its coefficients described ME used for total heat production (**HP**) and growth. A total of 630 pullets were randomly and equally assigned to 2 treatments: precision feeding (**PF**) and conventional skip-a-day feeding (**CON**) from 10 to 23 wk of age. The PF system allowed birds to enter voluntarily at any time, weighed them, and provided access to feed for 60 s if their body weight (**BW**) was less than the target BW. Birds in the CON treatment were fed as a group on alternate days. Energetic efficiency of pullets was evaluated using residual total heat production (**RHP**), defined as the difference between observed and predicted total HP. Additionally, ME intake (**MEI**), average daily gain (**ADG**), HP, and cumulative feed conversion ratio (**FCR**) were calculated for the entire experimental period. The energy partitioning model ($P < 0.05$) predicted MEI = $(120+u)\ BW^{0.68} + 1.52\ (ADG) + \varepsilon$. Total HP was (120 kcal/kg$^{0.68}$ + u); the energy requirement for each g of BW gain was 1.52 kcal/d. The random variable $u \sim N(0, \sigma_u^2)$ indicated a pen level HP standard deviation σ_u = 12.1 kcal/kg$^{0.68}$. Over the experimental period, for CON and PF treatments, respectively, MEI was 194 and 174 kcal/d ($P < 0.001$); ADG was 15.3 and 15.4 g/d ($P = 0.94$); HP was 129 and 111 kcal/kg$^{0.68}$ ($P < 0.001$); FCR was 4.888 and 4.057 ($P < 0.001$); and RHP was 0.12 and –0.12 kcal/kg$^{0.68}$ ($P = 0.73$). The CON pullets had similar ADG, but higher MEI relative to PF, consistent with levels of heat production predicted by RHP. The PF pullets had lower cumulative FCR compared to CON pullets. The PF pullets lost less energy as heat, likely because they were fed continuously, reducing the need to store and mobilize

nutrients compared to CON pullets. Thus, increased feeding frequency likely increased PF pullet efficiency.

Key words: precision livestock feeding, caloric restriction, energy partitioning, maintenance requirement, residual feed intake

3.2 Introduction

In the past decades, residual feed intake (**RFI**) has been used in animal nutrition studies as a biological estimate of feed utilization efficiency (Aggrey and Rekaya, 2013). Residual feed intake is an efficiency indicator that can account for variations in maintenance requirements and growth (Koch et al., 1963). Residual feed intake is the difference between observed and expected ME intake (Romero et al., 2009a). Metabolizable energy intake models can be used to estimate RFI as an efficiency indicator. The error term of ME intake models is referred to RFI. Moreover, ME intake models have been used to determine ME requirements for broiler breeders (Sakomura et al., 2003; Sakomura, 2004; Pishnamazi et al., 2015). Coefficients in these models are used to describe ME requirements for maintenance (**ME_m**), which is equivalent to total heat production (**HP**), and growth and egg production (retained energy) of broiler breeders. Residual maintenance ME requirement (**RME_m**) is another energetic efficiency factor and is defined as the difference between observed and expected ME_m (Romero et al., 2011). Residual maintenance ME requirement is calculated by the residual of the linear relationship between total HP and MEI (Romero et al., 2009a). Since the ME_m is the sum of all unretained ME, it is the same as total HP (NRC 1981; Zuidhof, 2018); RME_m can also be termed residual total HP (**RHP**). It is important to control feed intake (including energy intake) of broiler breeders during rearing to reduce reproductive problems during the production phase (Richards et al., 2010). Various methods have been used to restrict feed intake of broiler breeders (Mench, 2002) to optimize their body weight

(**BW**) for reproductive performance (Renema and Robinson, 2004). Skip-a-day feeding and limited everyday feed restriction methods are practical, and therefore commonly used by the broiler breeder industry. In the skip-a-day program, a feed allotment for 2 d is combined and fed to broiler breeders every second day. When feed intake rate of the most aggressive birds slows or they leave the feeder, the amount of feed remaining is sufficient for less aggressive birds to receive an adequate amount of feed. It was hypothesized that reducing competition for feed by providing more feed less frequently might improve BW uniformity (Bartov et al., 1988). Skip-a-day feeding increased flock uniformity relative to everyday feeding during severe feed restriction (de Beer and Coon, 2007). Moreover, it was observed that daily fed broiler breeders were more efficient compared to skip-a-day birds due to consistency in the supply of nutrients (de Beer et al., 2007; Zuidhof et al., 2015). On the other hand, overdrinking and stereotypic pecking are the result of feed restriction in broiler breeders (Hocking et al., 1997) and it brings up the concern that welfare of broiler breeder pullets may be compromised during rearing (Savory et al., 1993; Tolkamp et al., 2005). To allocate feed of broiler breeders they are typically weighed weekly (once or twice) and feed allocation decisions are based on their BW and rate of gain. However, a precise feed allocation requires weighing birds frequently, which requires labor (Schneider et al., 2005) or automated weighing equipment. Both of these increase the cost of hatching egg production. Although a major concern with automated data systems is the cost, it is conceivable that automation might eventually increase profitability through increased chick production, which in many parts of the world is currently well below broiler breeders' genetic potential. To the best of our knowledge, there has not been a practical method to weigh broiler breeders and allocate the required amount of feed based on their BW in real time. To provide a more stable energy balance and consistency in the supply of nutrients in broiler breeders, a novel precision feeding system was developed at the

University of Alberta (Zuidhof et al., 2016, 2017). The precision feeding system weighs individual broiler breeders and makes decisions in real time about whether or not to feed them after comparing their observed BW with their target BW. The implications of such a feeding approach for energy efficiency in broiler breeders have not been explored previously. The first objective of the current study was to develop a model whose coefficients describe ME cost for total HP and growth in broiler breeder pullets. Most previous literature assessed partitioning ME intake during lay (Rabello et al., 2006; Reyes et al., 2011). However, partitioning ME intake by modern broiler breeders has not been assessed as comprehensively during the rearing period. Additionally, using an energy partitioning model to measure energetic efficiency has not been studied thoroughly. The second objective was to evaluate energy efficiency and feed conversion rate of broiler breeder pullets fed using precision feeding and skip-a-day feeding systems. Efficiency was calculated using RFI, RHP, and feed conversion ratio (**FCR**). It was hypothesized that precision-fed broiler breeder pullets would be more efficient compared to pullets fed using conventional skip-a-day feeding method because of a higher feeding frequency.

3.3 Materials and Methods

3.3.1 Experimental Design

All procedures in the present study were approved by the Animal Care and Use Committee for Livestock at the University of Alberta. A total of 630 Ross 308 broiler breeder pullets were reared using pan feeders from 0 to 9 wk of age in floor pens. From 10 to 23 wk of age, they were randomly allocated to 2 treatments (7 pens of 45 birds in each treatment): precision feeding system (**PF**) and conventional skip-a-day feeding (**CON**) in a randomized complete block design. Seven environmentally controlled chambers (blocks) were used, and each block contained 1 replicate pen of each treatment.

3.3.2 Precision and Conventional Feeding Treatments

Birds in the PF treatment were fed using 1 feeding station per pen, the design and function of which are fully disclosed elsewhere (Zuidhof et al., 2016, 2017). Briefly, each PF pullet was identified by a unique radio frequency identification tag and weighed by a built-in platform scale when she entered the PF station. If her BW was equal to or greater than the target BW, the PF pullet was gently ejected by the station. However, if her BW was lower than the target BW, the PF station provided access to approximately 25 g of feed for 1 min, after which the pullet was ejected from the station. The feeder was mounted on a load cell so that feed could be weighed. Before feeding the feed was weighed and after feeding the remaining feed was weighed again. Feed intake was calculated as the initial minus the final feed weight. After weighing, the feeder was topped up to provide approximately 25 g of feed for the next feeding bout. For each visit, radio frequency identification, BW, and initial and final feed weight data were recorded in a database with a date and time stamp. Target BW was interpolated hourly for the PF treatment. Monochromatic green LED lights (525 nm wavelength) were mounted above the entry door and the feeder with a light intensity of 1.9 lux at the position of feeder. The intensity was measured using a light meter in all experimental pens during the scotophase when the only source of light was from the LED light of the PF station. The wavelength was strategically chosen to help pullets to enter the feeding stations and see feed during the scotophase without stimulating hypothalamic photoreceptors (Rodriguez, 2017). Thus, PF pullets could access feed 24 h/d and sequentially received several small meals over a full 24 h/d rather than 1 large meal. During the first 3 wk of the study (10, 11, and 12 wk of age), PF pullets required training to become familiarized with the feeding stations. They had to learn that they would receive a small meal as a reward for voluntarily entering the feeding station. Daily during week 10, pullets were encouraged to enter PF system

voluntarily, by guiding them to the feeder inside the station. The PF pullets that were slower to learn to use the PF stations were identified from the database records by low visit frequencies. These birds were remedially trained every second day during week 11 and 12. During this time, all PF pullets with BW less than 70% of their target BW were trained. Training continued or was reinstated when pullets voluntarily entered the PF station less than 4 times for 2 consecutive days or when the BW of pullet was less than 70% of the target BW. It was decided a priori to remove any PF birds from the experiment that were not able to learn the principle of individual feeding by the end of week 12. However, all birds were able to learn to use the PF stations. Thus, none of the PF birds were removed from the experiment. Because the initial BW of the largest birds exceeded the target BW, and to ensure that heavy PF pullets would receive feed and not be immediately ejected, the BW of the heaviest PF pullet was initially assigned as the PF target BW. This BW was maintained as the target BW until 13 wk, after which an hourly interpolated breeder-recommended Ross 308 BW target (Aviagen, 2011) was adopted. In the CON treatment, pullets were fed on alternate mornings (skip-a-day feeding). Feed allocation for the CON treatment was based on weekly BW recording, to maintain breeder recommended BW targets. At the end of each week in the morning, all pullets in both treatments were individually weighed manually. The CON pullets were weighed before feeding and the PF pullets were weighed while PF stations were working. The rationale of the PF system was to provide continuously a small amount of feed to the PF pullets throughout the day. Thus, the PF stations were not shut down before weighing birds to avoid ceasing the continuous feeding of the PF pullets. There was a small amount of feed in the gut of PF pullets, which would have had a minimal effect on their BW. The average daily gain (**ADG**) was calculated for each experimental unit (pen). The cumulative FCR for each experimental unit was calculated weekly by dividing the total feed intake to the total ADG.

3.3.3 Management

Each experimental pen contained pine shavings as litter at a depth of approximately 5 cm. Two suspended nipple drinkers (3.2 pullets per nipple) provided water ad libitum throughout the experiment. The stocking density was 5.4 birds/m^2. There were 3 round hanging feeders in each CON pen, providing 10 cm of feeder space per pullet. The feeder in each PF station was 4.8 cm wide, and only 1 bird was provided access at a time. Temperature was 20.8 ± 0.34°C during the entire experimental period. Photoperiod was 10L:14D with a light intensity of 10 lux. A single broiler breeder grower diet (Table 3-1) was formulated according to breeder recommendations (Aviagen, 2013) and provided in pellet form to all treatments for the duration of the study.

3.3.4 Diet Composition

The apparent metabolizable energy (**AME**) content of the diet was determined by adding 2% acid-insoluble ash marker (Celite, Celite 281, Lompoc, CA) for 4 d in the diet at both 16 and 23 wk of age. Two birds in each pen (14 per treatment) were randomly selected at 16 and 23 wk of age and euthanized by cervical dislocation 4 h after the CON treatment birds were fed. Ileal digesta samples were collected by gently squeezing the intestinal tract from Meckel's diverticulum to the ileal-cecal-colon junction. Digesta samples were pooled for each experimental pen, and stored at –20°C until analysis. Samples were later oven dried at 60°C for 48 h and ground prior to analysis. Insoluble ash content of diet and digesta samples was determined. Briefly, acid insoluble ash remained after digesting the digesta samples with 4 N HCL and ashing the residue at 500°C (Vogtmann et al., 1975). Gross energy (**GE**) was measured using bomb calorimetry for feed and digesta samples. The AME values were calculated using the following equation (Scott and Boldaji, 1997):

$$\text{AME} = \text{GE}_{\text{feed}} - \text{GE}_{\text{digesta}} \times \frac{\text{Marker}_{\text{feed}}}{\text{Marker}_{\text{digesta}}}$$

where GE = gross energy (kcal/kg of sample) and Marker = concentration of acid insoluble ash in the sample. After AME determination, a 2% correction was applied to the analyzed AME values for each treatment to correct AME to the energy content of the diet that was free of the acid insoluble ash marker. The AME values were not corrected for nitrogen retention. Apparent ME values were expressed on an as fed basis. Nitrogen content was determined by the combustion method using a Leco TruMac N determinator (Leco Corporation, St. Joseph, MI), and CP was estimated using a factor of 6.25.

3.3.5 Carcass Traits

At 16 and 23 wk of age, 28 birds per treatment were euthanized and breast muscle and fat pad weights were recorded. Fat pad weight and breast muscle weight as a percentage of live BW were calculated for both treatments.

3.3.6 Statistical Analysis

3.3.6.1 Analysis of Variance

The nonlinear ME intake model was fit for the overall experimental period from 10 to 23 wk of age using the NLMIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). Linear regression between (a+u) and ME intake was conducted using the MIXED procedure. Treatment effects for RFI and total HP (a + u) were subsequently evaluated using the MIXED procedure as a 1-way ANOVA. Treatment effects for ME intake, BW, ADG, cumulative FCR, partitioning of ME intake toward total HP and BW gain, and carcass traits were evaluated using 2-way ANOVA using the MIXED procedure in SAS, with age and treatment as sources of variation. The PDIFF

option of the LSMEANS statement used to estimate pairwise differences between means and differences were reported where $P \leq 0.05$. Trends were considered where $0.05 < P \leq 0.10$.

3.3.6.2 ME Partitioning Model

A nonlinear mixed model (Eq. 1) was used to derive ME requirements for total HP and gain of broiler breeder pullets from 10 to 23 wk of age:

$$\text{Observed MEI} = \text{Expected MEI} + \varepsilon$$

$$\text{Expected MEI} = (a + u) \times BW^b + c \times ADG$$

$$\text{Observed MEI} = (a + u) \times BW^b + c \times ADG + \varepsilon \quad [1]$$

where MEI was ME intake (kcal/d); a was ME_m or the average total HP for all pens for the entire experimental period (kcal/kg^b); $u \sim N(0, \sigma_u^2)$ (kcal/d) was the pen-associated deviation from a such that $a + u_i$ was the total HP estimate for each unique experimental pen i; BW was the average BW (kg) of each experimental unit during each week, which was used to estimate total HP; BW^b was metabolic BW; c (kcal/g) was the coefficient of ADG (g/d) that defined the ME cost for each g of BW gain; and ε was the residual or unexplained error (RFI; kcal/d). From 10 to 23 wk of age, the percentage of MEI partitioned to ADG (G_p) and the percentage of MEI partitioned to total HP (HP_p) were calculated weekly as:

$$G_p = \left[\frac{c \times ADG}{\text{Observed MEI}}\right] \times 100\ \%$$

$$HP_p = \left[\frac{[a + u] \times \text{Metabolic BW}}{\text{Observed MEI}}\right] \times 100\ \%$$

3.3.7 Residual Total HP

The relationship between total HP and ME intake from 10 to 23 wk of age was estimated by a linear regression (2), modified from Romero et al. (2009b) such that ME intake was expressed per unit of metabolic BW:

$$E\ (a+ u) = \text{intercept} + \text{slope} \times MEI + \varepsilon \quad [2]$$

where E (a+ u) was the total HP coefficient estimated for each pen (kcal/kg^b); slope (kcal/kg^b/kcal/kg^b) was the coefficient defining the linear rate of change of total HP with respect to ME intake (MEI, kcal/kg^b); and ε was the RHP (kcal/kg^b). On the resulting graph (see Figure 3-1), RHP is represented by the vertical distance between every point and the regression line. Since the units for the slope coefficient cancel, the slope can be directly interpreted as the proportion of dietary energy lost by the pullets as heat.

3.4 Results and Discussion

3.4.1 ME Intake

Overall, ME intake increased from 148 at wk 10 to 196 kcal/d at wk 23 ($P < 0.001$, Table 3-2). The BW of pullets also increased from 1,005 to 2,517 g from 10 to 23 wk of age ($P < 0.001$, Table 3-3). Moreover, ADG increased from 10.7 to 23.1 g from 10 to 23 wk of age ($P < 0.001$, Table 3-4). Overall ME intake for both treatments increased from 10 to 23 wk of age primarily because BW increased with age and broiler breeder pullets needed more ME to meet their maintenance, and ADG requirements; also, ADG increased with age, for which ME was required. The feeding frequency and the meal size for the PF pullets was 8.82 ± 0.41 meals per day, and 7.76 ± 0.12 g/meal respectively. In contrast, the feeding frequency for the CON pullets was 0.5 meals per day since they were fed on alternate days and their meal size was 75.18 ± 1.2 g/d on average from 10 to 23 wk of age.

The ME requirement for broiler breeders has been defined as that required for maintenance, gain, and egg production (Sakomura, 2004). Since there is no egg production in rearing period of broiler breeders. Thus, pullets partition ME to maintenance and BW gain. Since ME_m is equal to total HP (Zuidhof, 2018), ME intake is partitioned to total HP and growth during the rearing period

of broiler breeder pullets. Metabolizable energy intake was reported as 155 and 316 kcal/d for broiler breeder pullets at 10 and 23 wk of age, respectively (Sakomura, 2004). At 20 wk of age, ME intake was estimated 270 kcal/d for broiler breeder pullets (Pinchasov and Galili, 1990). In the present study, the average ME intake of broiler breeder pullets from 10 to 12 wk of age was 147 and from 20 to 23 wk of age was 241 kcal/d. This suggests that ME intake of pullets in 2017 has reduced gradually since 1990. There are 2 main reasons for this. The first relates to changes in broiler breeder body composition. Broilers in 2005 had greater breast muscle weight and yield compared to birds in 1957, whereas abdominal fat pad decreased from 1957 to 2005 due to commercial selection pressure (Zuidhof et al., 2014). The selection pressure has also affected the body composition of broiler chicken parent stocks. Broiler breeders in 1980 had lower breast muscle percentage compared to the broiler breeders in 2000 (14.9% vs. 21.2%) and higher fat pad percentage (5.4% vs. 2.7%) due to genetic selection for broiler productivity traits (Eitan et al., 2014). Increasing breast muscle weight in modern broiler breeders indicates that protein deposition is increased. Lean tissue is composed of protein (5.5 kcal/g; Pullar and Webster, 1977; Leeson and Summers, 2001; Romero et al., 2014) and water (0 kcal/g) deposition in the body is less energetically expensive compared to fat deposition, which requires approximately 9.2 kcal/g (Johnston, 1970; Leeson and Summers, 2001; Romero et al., 2014; Zuidhof et al., 2014). A reduction in ME intake over time is consistent with highly feed restricted modern broiler breeder pullets depositing relatively more lean tissue and less adipose tissue. The second reason that ME intake was reduced may be related to increased efficiency. Although the growth potential of broilers increased by more than 450% from 1957 to 2005, FCR to 56 d of age has decreased to 1.918 in 2005 from 2.854 in 1957 (Zuidhof et al., 2014). Moreover, modern broilers have greater gut mass compared to unselected birds, which increases digestion, and absorption in modern

broilers relative to unselected ones (Jackson and Diamond, 1996). It follows that body composition changes and increased efficiency in modern broiler breeder pullets resulted in their lower ME intake compared to the pullets in the 2000s and in the 1990s. From 10 to 12 wk of age, while PF pullets were being familiarized with the feeding station, the target BW was set to the weight of the heaviest bird. During this time, most of the PF birds were allowed to eat more than the CON treatment birds. Much of this occurred during the first week of the study. During week 10, PF pullets consumed 42% more ME compared to CON pullets ($P < 0.05$, Table 3-2). The overall average daily ME intake for PF treatment (174 kcal/d) was 90% of the CON treatment (194 kcal/d; $P < 0.001$, Table 3-2). Similarly, but to a lesser degree, ME intake of daily-fed pullets (15,204 kcal/bird; 159 kcal/d) from 15 to 22 wk of age was 97% of ME intake in skip-a-day broiler breeder pullets (15,729 kcal/bird; 164.5 kcal/d; Zuidhof et al., 2015). Daily-fed broiler breeder hens fed based on skip-a-day feeding on rearing period had around 70 kcal/d higher feed intake compared to hens fed based on daily restricted method during rearing and laying periods to reach the same BW (de Beer and Coon, 2007). Skip-a-day feeding can reduce metabolic efficiency because broiler breeder pullets need to store and mobilize greater amounts of nutrients constantly compared to daily fed (Richards et al., 2010). Deposition and mobilization of nutrients are not completely efficient processes (McCue, 2006; de Beer and Coon, 2007) and lower efficiency of skip-a-day fed broiler breeders compared to daily fed broiler breeders can be attributed to repeated storage and mobilization of nutrients (de Beer and Coon, 2007). Increased feeding frequency in PF pullets could have increased their efficiency, thereby requiring a lower ME intake to photostimulation age compared with the CON treatment. The first priority of animals is to satisfy the maintenance energy requirement, after which energy can be partitioned to growth and egg production (Pishnamazi et al., 2015). Although PF hens had lower ME intake compared to CON hens during

the entire experimental period, they had greater BW relative to CON birds (Table 3-3). The CON and PF pullets in the present study partitioned 89.0 vs. 86.9% of the ME intake into total HP, respectively, during the entire experimental period (P = 0.020, Table 3-5). Conversely, birds in the CON and PF treatments partitioned 11.0 and 13.1% of ME intake into BW gain (growth), respectively (P = 0.02, Table 3-5). The results of the present study suggest that CON and PF hens partitioned ME intake in different proportions, which may indicate differences in their underlying metabolism.

3.4.2 Growth

Both treatments were grown using the same target BW until 23 wk of age, with the exception that from 10 to 12 wk of age the target BW for PF pullets was higher in the PF treatment to ensure the even the heaviest birds had access to feed. There was no treatment difference in mortality (4.1% in both treatments; data not shown). Weekly and overall ADG did not differ between PF and CON pullets from 10 to 23 wk of age (Table 4). It was reported that ADG was 13 and 16 g/d for broiler breeder pullets from 9 to 14 and 15 to 20 wk of age, respectively (Sakomura et al., 2003). Moreover, ADG was 14 g/d from 3 to 20 wk of age (Pinchasov and Galili, 1990). These values were similar to the 15 g/d reported in the current study. This indicated that the target BW of broiler breeders have undergone few changes during the past 29 yr relative to large increases in growth potential.

3.4.3 Carcass Traits

There was no treatment effect on breast muscle as a percentage of BW at 16 or 23 wk (Table 3-6). There was no significant treatment difference in fat pad percentage at 16 wk of age; however, in spite of having lower BW at 23 wk, pullets in the CON treatment had a 1.4-fold increase in relative fat pad weight compared with the PF treatment (Table 3-6). Skip-a-day broiler

breeder pullets had 10% greater fat pad weights compared to daily fed pullets (37.4 g vs. 33.6 g), 11% less pectoralis major weight (395.0 g vs. 444.3 g), and 10% less pectoralis minor weight (122.3 g vs. 135.5 g) at 22 wk of age ($P < 0.05$; Zuidhof et al., 2015). It was suggested that repeated long durations of negative energy balance in skip-a-day pullets conditioned them to partition more energy into storage in the abdominal fat pad (Zuidhof et al., 2015). In pullets fed skip-a-day, expression of genes involved in lipogenic activity in the liver, such as acetyl– coenzyme A carboxylase, malic enzyme, and fatty acid synthase, were increased compared to daily fed birds (de Beer et al., 2007). Moreover, excess dietary energy during feeding is converted to triglycerides, which are stored in adipose tissues (de Beer et al., 2007). Fasting alters metabolism in broiler breeders, increasing lipid mobilization and plasma free fatty acid levels to provide required energy (Richards et al., 2010). During fasting, the body needs to mobilize fatty acids from adipose tissues and glucose from liver glycogen (de Beer et al., 2007). In skip-a-day programs, more short-term nutrient storage and subsequent mobilization are needed compared with a daily feeding program (Richards et al, 2010) to maintain homeostasis. After depleting liver glycogen, catabolism of muscle proteins meets the energy requirements for the body through gluconeogenesis (Robert et al., 2003). After feeding, more glucose is available from feed. Some is used to meet energy requirements immediately, some is stored in the liver as glycogen and some is stored in muscles, and the rest is converted via lipogenesis and stored as fat (Robert et al., 2003). Lipogenesis starts with acetyl-CoA, a molecule that is formed from the metabolism of glucose (Robert et al., 2003). Surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA are converted to fat through lipogenesis during the anabolic phase of the feeding cycle (Robert et al., 2003). In the current experiment, the CON treatment had a lower feeding frequency (once per 48 h) and therefore greater temporal variation in glucose availability, requiring more storage and

mobilization of nutrients, which probably provided more substrate for lipogenesis in CON birds. The CON pullets had higher ME intake compared to PF pullets, which indicates that they had more total energy available; however, they lost more energy as heat relative to PF pullets (discussed further in the total HP section).

3.4.4 ME Partitioning Model

The quantitative model (3) describing ME partitioning in both treatments was ($P < 0.001$):

$$MEI = (120 + u) \times BW^{0.68} + 1.52 \times ADG + \varepsilon \quad [3]$$

$$u \sim N(0, \sigma_u^2); \sigma_u = 12.1;$$

$$e \sim N(0, \sigma_e^2); \sigma_e = 40.6$$

The MEI was ME intake (kcal/d); coefficient a = 120 indicates that total HP averaged 120 kcal/$BW^{0.68}$; the coefficient c = 1.52 estimates that broiler breeder pullets needed 1.52 kcal of ME per g of BW gain. The SD for total HP (σ_u) was 12.1 kcal/$kg^{0.68}$ and the SD for the residual term (σ_e) was 40.6 kcal/d. The SD for total HP was 22.8 and σ_e = 6.6 kcal/$kg^{0.75}$ in broiler chickens (Zuidhof et al., 2014). The SD for total HP was 21.3 for broiler breeders whose feed was allocated on a group basis from 20 to 60 wk of age, and σ_u = 58.3 kcal/$kg^{0.54}$ when feed was allocated on an individual basis (Romero et al., 2009a). The analysis in the current study was conducted at pen level, which may explain the lower variance compared with Romero et al. (2009a).

3.4.5 The ME Requirement for BW Gain

The ME cost for BW gain (**ME_g**) in the current study was 1.52 kcal/g. This is much lower than reported by Sakomura et al. (2003), who estimated ME_g to be 2.50 kcal/g from 9 to 14 wk, and 3.24 kcal/g from 15 to 20 wk in two age-specific ME requirement models developed for Hubbard Hi-Y broiler breeder pullets using a factorial method. These researchers used a linear model to estimate ME_g, in contrast with the nonlinear model used in the current study. It is possible

that ME_g estimates might differ in part due to modeling methodology but may also vary due to differences in strain and body composition. Age-related changes in the chemical composition of growth can affect ME requirement for growth. Protein contains 5.5 kcal/g (Pullar and Webster, 1977; Chwalibog, 1991), and lean tissue is made up of 75% water (0 kcal/g) and 25% protein (Claus and Weiler, 1994). Thus, assuming lean tissue contains primarily water and protein and without considering any total HP as a byproduct of formation, the retained energy for each g of lean tissue is approximately 1.38 kcal/g. The coefficient c = 1.52 kcal/g estimated in the current experiment is very close to the value of retained energy for each gram of lean tissue and it is consistent with the observation that modern broiler breeders deposit primarily lean tissue while under conventional feed restriction. Because of continued selection for broiler traits, modern broiler breeder pullets deposit less fat and may require less energy for BW gain compared to broiler breeders in 2003 (Sakomura et al., 2003).

3.4.6 Total HP

The ME_m includes energy required for basal metabolism, thermal regulation, and activity (NRC, 1981; Emmans, 1994) and since this energy is lost as heat, ME_m is equal to total HP (Zuidhof, 2018). Total HP of broiler breeders raised on the floor was 20% higher than those raised in cages (Sakomura, 2004). Physical activity accounted for 20% of total HP (Wenk, 1997). Temperature can also affect total HP. Total HP in broiler breeder hens decreased in a quadratic manner with increasing temperature to 24.3℃, after which total HP increased (Pishnamazi et al., 2015). There was a positive relationship between feed intake and total HP in broiler breeder pullets (R^2 = 0.95; Pishnamazi et al., 2008). Body composition can also influence total HP. For example, body fat has a lower maintenance cost than body protein (Close, 1990). Total HP in broiler breeder pullets was higher than in hens because mature birds have a higher proportion of fat compared to

protein in their bodies (Sakomura, 2004). Moreover, higher total HP in growing animals was due to high energy demand for protein synthesis (Blaxter, 1989). In the current experiment, total HP of CON pullets was 16% higher than PF pullets (129 and 111 kcal/kg $BW^{0.68}$, respectively; $P < 0.001$; Table 3-5). However, CON pullets had greater fat pad percentage at 23 wk of age compared to PF pullets and CON pullets had higher total HP compared to PF pullets. Still, the CON pullets had higher total HP compared to PF pullets, and a higher ME intake (194 vs. 174 kcal/d, respectively). Thus, one factor contributing to higher total HP by CON birds is diet-induced thermogenesis. The slope of a regression between total HP and ME intake quantifies this (Figure 3-1). In the current study 75% of every additional kcal of ME consumed was lost as heat. This will be discussed further in the next section. Behavioral observations from the current experiment showed that although PF pullets had 1.6-fold higher incidence of aggressive behaviors (12.7 vs. 8.0 per 15 min) compared with CON pullets ($P = 0.001$; Girard et al., 2017), they walked and stood 7% less than CON pullets (Gilmet, 2015). Thus, CON pullets had higher physical activity compared to PF pullets. No observations were made during CON feeding time, which was likely the time when CON birds had much higher rates of activity and aggression. Thus, physical activity may have also contributed to higher total HP of CON compared with PF treatment pullets.

3.4.7 Residual Total HP

Regression analysis using combined PF and CON treatment data defined the relationship between expected total HP (E(a+u)) and ME intake (MEI; $P < 0.001$; Figure 3-1):

$$E(a+u) = 1.56 + 0.87\ MEI \quad [4]$$

This result suggests that total HP increases linearly with increasing ME intake. Since the units for the slope coefficient cancel, the slope can be directly interpreted as the proportion of ME intake that is lost as heat. Specifically, the model predicts that 87% of ME consumption by broiler breeder

pullets from 10 to 23 wk of age was lost as heat. This linear regression model (Eq. 4) was initially defined by Romero et al. (2009b) to account for intake-related changes in total HP, which was the main shortcoming of previous mathematical models. Those authors reported an apparently lower rate of total HP (0.34 kcal/$kg^{0.54}$/kcal consumed) compared to the current study, for broiler breeder hens from 20 to 60 wk of age. When ME intake is corrected to metabolic BW, this translates to a total HP of 25% early in production to 15% later in the laying phase. Since feed restriction is less severe during the laying phase, lower total HP would be expected compared with the pullet phase. By changing the units of the independent x-axis variable to intake per unit of metabolic BW, interpretation of the result directly as efficiency of dietary energy use is simplified. It was reported earlier that PF and CON treatments partitioned 86.9 and 89.0%, respectively, of ME intake toward total HP. The estimate of 87% thus seems a reasonable estimate. It was estimated that 70 to 88% of ME intake partitioned to total HP in broiler breeder pullets from 5 to 20 wk of age (Sakomura et al., 2003). This value appears to have increased with further selection for broiler traits.

3.4.8 Energetic Efficiency

In the current study, RFI was not affected by treatment at the $P < 0.05$ level (–3.8 and 3.7 kcal/d for PF and CON pullets respectively; $P = 0.064$; Table 3-5). Residual feed intake was estimated by deducting expected ME intake (for total HP + ADG) from the observed ME intake. Differences in the composition of BW gain affected RFI (Basarab et al., 2003). Inefficient steers with high RFI had higher ME intake, higher HP ($P < 0.01$), and greater carcass fat ($P < 0.05$) compared to efficient steers (Basarab et al., 2003). The higher ME intake of inefficient steers was due to an increase in the ME_m and heat increment of feeding (Basarab et al., 2003). The variation in feed efficiency of high and low RFI steers was due to differences in fat deposition of internal organs and energy requirements (Gomes et al., 2012). The increase in carcass fat of the CON

treatment (0.5% of live BW) in the current experiment (Table 3-6) may have been insufficient to identify a significant difference in RFI. In the current experiment, although CON pullets had higher ME intake compared to PF pullets, they did not have greater BW or ADG compared with PF hens. Moreover, CON pullets had higher total HP (89% of ME intake) relative to PF pullets (86.9% of ME intake) due to temporal variation in ME supply, creating conditions that required storage and mobilization of nutrients. Thus, CON pullets were less efficient than PF pullets. Decreased energetic efficiency of laying hens (estimated using RFI) was due to higher feed intake, which indicated that RFI was impacted by heat increment of feeding (Swennen et al., 2007). However, it is important to note that, in most cases, high-producing animals have higher feed intake and RFI can penalize the extra feed intake. Residual maintenance ME (RHP in the current study) can estimate energetic efficiency without being confounded by feed intake (Romero et al., 2009a). Residual HP is estimated from the residual of a linear regression between total HP (a + u) and ME intake, and it indicates heat loss in a way that is unbiased by ME intake. It is, therefore, an estimate of relative efficiency after considering not only energy requirements for maintenance and retained energy but also for feed intake. The points below the regression line in Figure 3-1 indicated the more efficient pens and the points above the regression line indicated the less efficient pens. Residual HP did not differ between CON and PF treatments (P = 0.73; Table 3-5). Although the CON hens had higher ME intake (194 kcal/d) compared to the pullets in PF treatment (174 kcal/d) and partitioned more energy toward total HP (89.0% of the observed ME intake) relative to PF pullets (86.9% of the observed ME intake), they did not lose more energy daily per unit of metabolic BW. Thus, treatment differences in body composition and feeding frequency-related differences in nutrient mobilization and storage likely contributed to differences in efficiency between treatments in the current study.

3.4.9 Feed Conversion Ratio

In the current experiment, cumulative FCR to 23 wk in the PF treatment was 83% of FCR in the CON treatment (Table 3-7). Feed conversion ratio was lower in 3 daily fed treatments (standard diet, scatter feeding, and BW grading) compared with skip-a-day pullets (Zuidhof et al., 2015). In addition, it was reported that FCR in daily-fed pullets at 21 wk of age was 95% of the FCR of skip-a-day fed pullets (de Beer and Coon, 2009). Thus, research has consistently shown that birds with higher feeding frequency grow more efficiently compared to skip-a-day-fed birds. Although FCR is a good indicator of efficiency, it may not be the best one. Residual feed intake is a biological indicator of energetic efficiency and although it is more difficult to calculate, it is a more precise indicator of efficiency compared to FCR because it accounts for maintenance (total HP), BW gain, and egg production (where relevant) energy requirements.

On the other hand, RFI may not be the best indicator of the energetic efficiency of biological processes. Residual feed intake is biased by differences in feed intake levels (Gabarrou et al., 1998). Residual HP is relatively new indicator of energetic efficiency. Although RHP is more complicated to calculate compared to RFI, it is to date the most precise indicator of efficiency at a biological level because it estimates the energy losses (as heat) without being biased by energy levels. According to the current analysis, the biological efficiency of the pullets in both treatments was similar. Thus, differences in efficiency can be attributed to energy using processes such as nutrient storage and mobilization, or fat deposition, which were likely managed similarly by birds in both treatments in the current study.

3.5 Conclusions

In conclusion, CON pullets had lower BW, despite a higher ME intake and higher total HP compared to PF pullets. The PF pullets had lower cumulative FCR compared to CON pullets; thus,

PF pullets used their feed more efficiently. However, residual HP analysis suggests that both treatments had similar biological efficiency. The CON treatment pullets did not appear to lose more energy as heat as a result of diet induced thermogenesis, but because they were fed less frequently, probably used the extra energy they consumed to store and mobilize nutrients, and to deposit fat in their body.

3.6 Acknowledgments

Financial support from Alberta Livestock and Meat Agency (Edmonton, Alberta, Canada), Alberta Innovates Bio Solutions (Edmonton, Alberta, Canada), Agriculture and Food Council (Edmonton, Alberta, Canada), Alberta Chicken Producers (Edmonton, Alberta, Canada), Poultry Industry Council (Guelph, Ontario, Canada), Danisco Animal Nutrition (DuPont; Marlborough, Wiltshire, United Kingdom), Canadian Hatching Egg Producers (Ottawa, Ontario, Canada), Alberta Hatching Egg Producers (Edmonton, Alberta, Canada), and Ontario Broiler Chicken Hatching Egg Producers Association (Guelph, Ontario, Canada) is gratefully acknowledged. In kind technical support was provided by Xanantec Technologies, Inc. (Edmonton, Alberta, Canada), excellent technical expertise provided by staff and students at the University of Alberta Poultry Unit (Edmonton, Canada), and base supporters of the Poultry Research Centre (Edmonton, Canada) are also gratefully acknowledged.

3.7 References

Aggrey, S. E., and R. Rekaya. 2013. Dissection of Koch's residual feed intake: Implications for selection. Poult. Sci. 92:2600–2605.

Aviagen, 2011. Ross 308 Parent Stock: Performance Objectives. Aviagen Inc., Huntsville, AL. Accessed Apr. 2015. http://en.aviagen.com/assets/Tech_Center/Ross_PS/Ross- 308-PS-PO-2011.pdf.

Aviagen. 2013. Ross 308 PS nutrition specifications. Aviagen Incorporated, USA. http://sedghi.iut.ac.ir/sites/sedghi.iut.ac.ir/files//files_course/ross308ps-nutritionspecs-2013final.pdf. Accessed November 2016.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83:189–204.

Bartov, I., S. Bornstein, Y. Lev, M. Pines, and J. Rosenberg. 1988. Feed restriction in broiler breeder pullets: skip-a-day versus skip-two-days. Poult. Sci. 67:809–813.

Blaxter, K. L. 1989. The utilization of the energy of food. Page 254–336 in Energy Metabolism in Animal and Man. Cambridge Univ. Press, Cambridge, England.

Byerly, T. C., J. W. Kessler, R. M. Gous, and O. P. Thomas. Feed requirements for egg production. 1980. Poult. Sci. 59:2500–2507.

Chwalibog, A. 1991. Energetics of animal production-research in Copenhagen, review and suggestions. Acta. Agr. Scand. 41:147–160.

Claus, R, Weiler, U. 1994. Endocrine regulation of growth and metabolism in the pig: a review. Livest. Prod. Sci. 37:245–60.

Close, W. H. 1990. The evaluation of feeds through calorimetry studies. Pages 21-39 in J. Wiseman and D. J. A. Cole eds. Feedstuff evaluation. Butterworths, The University Press, Cambridge, UK.

de Beer, M. 2009. Current approaches to feeding broiler breeders. Pages 104-114 in Proc. 17th Eur. Symp. Poult. Nutr., Edinburgh, Scotland. World's Poult. Sci. Assoc., Attleborough, United Kingdom.

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927–1939.

de Beer, M., and C. N. Coon. 2009. The effect of different feed restriction programs and dietary L-carnitine supplementation on reproductive performance, efficiency, frame size and uniformity in broiler breeder hens. Int. J. Poult. Sci. 8:409–425.

de Beer, M., R. W. Rosebrough, B. A. Russell, S. M. Poch, M. P. Richards, and C. N. Coon. 2007. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. Poult. Sci. 86:1726–1738.

Emmans, G. C. 1994. Effective energy: a concept of energy utilization applied across species. Br. J. Nutr. 71:801–821.

Gabarrou, J. F., P. A. Geraert, N. François, S. Guillaumin, N. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. Br. Poult. Sci. 39:79–89.

Gilmet, T.E.R.M. 2015. Behavioural implications of precision feeding broiler breeders. MSc Diss. Univ. Alberta, Edmonton, Alberta.

Girard, T. E., M. J. Zuidhof, and C. J. Bench. 2017. Aggression and social rank fluctuations in precision-fed and skip-a-day-fed broiler breeder pullets. Appl. Anim. Behav. Sci. 187:38–44.

Goerzen, P. R., and W. L. Julsrud, and F. E. Robinson. 1996. Duration of fertility in ad libitum and feed-restricted caged broiler breeders. Poult. Sci. 75:962–965.

Gomes, R. C., R. D. Sainz, S. L. Silva, M. C. Ce´sar, M. N. Bonin, and P. R. Leme. 2012. Feedlot performance, feed efficiency reranking, carcass traits, body composition, energy requirements,

meat quality and calpain system activity in Nellore steers with low and high residual feed intake. Livest. Sci. 150:265–273.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500–1508.

Jackson, S., and J. Diamond. 1996. Metabolic and digestive responses to artificial selection in chickens. Evolution 50:1638–1650.

Johnston, D. W. 1970. Caloric density of avian adipose tissue. Comp. Biochem. Physiol. 34: 827–832.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22:486–494.

Leeson, S., and J. D. Summers. 2001. Energy. Pages 34–99 in Nutrition of the Chicken. 4th ed. Univ. Books, Guelph, ON, Canada.

Liu, L., X. Wang, H. Jiao, J. Zhao, and H. Lin. 2015. Glucocorticoids inhibited hypothalamic target of rapamycin in high fat diet-fed chicks. Poult. Sci. 94:2221–2227.

McCue, M. D. 2006. Specific dynamic action: a century of investigation. Compar. Biochem. Physiol. 144A:381–394.

Mench, J. A. 2002. Broiler breeders: Feed restriction and welfare. World's Poult. Sci. J. 58:23–29.

National Research Council. 1981. Nutritional energetics of domestic animals and glossary of energy terms. Second rev. ed. Natl. Acad. Press, Washington, DC.

Pinchasov, Y., and D. Galili. 1990. Research note: Energy requirement of feed-restricted broiler breeder pallets. Poult. Sci. 69:1792–1795.

Pishnamazi, A., R. A. Renema, D. C. Paul, I. I. Wenger, and M. J. Zuidhof. 2015. Effects of environmental temperature and dietary energy on energy partitioning coefficients of female broiler breeders. J. Anim. Sci. 93:4734–4741.

Pishnamazi, A., R. A. Renema, M. J. Zuidhof, and F. E. Robinson. 2008. Effect of initial full feeding of broiler breeder pullets on carcass development and body weight variation. J. Appl. Poult. Res. 17:505–514.

Pullar, J. D., and A. J. F. Webster. 1977. The energy cost of protein and fat deposition in the rat. Br. J. Nutr. 37:355–361.

Rabello, C. B. V., N. K. Sakomura, F. A. Longo, H. P. Couto, C. R. Pacheco, and J. B. K. Fernandes. 2006. Modelling energy utilisation in broiler breeder hens. Br. Poult. Sci. 47:622–631.

Renema, R. A., and F. E. Robinson. 2004. Defining normal: Comparison of feed restriction and full feeding of female broiler breeders. World's Poult. Sci. J. 60:508–522.

Renema, R. A., M. E. Rustad, and F. E. Robinson. 2007. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. Worlds Poult. Sci. J. 63:457–472.

Reyes, M. E., C. Salas, and C. N. Coon. 2011. Energy requirement for maintenance and egg production for broiler breeder hens. Int. J. Poult. Sci. 10:913–920.

Richards, M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMurtry. 2010. Feed intake regulation for the female broiler breeder: In theory and in practice. J. Appl. Poult. Res. 19:182–193.

Robert K. M., K. G. Daryl, A. M. Peter, W. R. Victor. 2003. Harper's Illustrated Biochemistry. Twenty-sixth edition. 2003 by the McGraw-Hill Companies Inc. Printed in London. pp. 12–21.

Rodriguez, A. 2017. Effects of daytime and supplemental light spectrum on broiler breeder growth and sexual maturation. MSc thesis. University of Guelph, Guelph, Ontario.

Romero., L. F., J. S. Sands, S. E. Indrakumar, P. W. Plumstead, S. Dalsgaard, and V. Ravindran. 2014. Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease. Poult. Sci. 93:2501–2513.

Romero, L. F., M. J. Zuidhof, R. A. Renema, A. Naeima, and F. E. Robinson. 2009a. Characterization of energetic efficiency in adult broiler breeder hens. Poult. Sci. 88:227–235.

Romero, L. F., M. J. Zuidhof, R. A. Renema, F. E. Robinson, and A. Naeima. 2009b. Nonlinear mixed models to study metabolizable energy utilization in broiler breeder hens. Poult. Sci. 88:1310–1320.

Romero, L. F., M. J. Zuidhof, R. A. Renema, A. Naeima, and F. E. Robinson. 2011. Effects of maternal energy efficiency on broiler chicken growth, feed conversion, residual feed intake, and residual maintenance metabolizable energy requirements. Poult. Sci. 90:2904–2912.

Sakomura, N. K. 2004. Modeling energy utilization in broiler breeders, laying hens and broilers. Braz. J. Poult. Sci. 6:1–11.

Sakomura, N. K., R. Silva, H. P. Couto, C. Coon, and C. R. Pacheco. 2003. Modeling metabolizable energy utilization in broiler breeder pullets. Poult. Sci. 82:419–427.

Schneider, B., M. Zuidhof, F. Robinson, and R. Renema. 2005. Allocating feed to female broiler breeders: technical bulletin#2. Technical Bulletin #2. Accessed Jan. 2018. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/pou9452.

Scott, T. A., and F. Boldaji. 1997. Comparison of inert markers [chromic oxide or insoluble ash (Celite^{TM})] for determining apparent metabolizable energy of wheat or barley–based broiler diets with or without enzymes. Poult. Sci. 76:594–598.

Swennen, Q., P.-J. Verhulst, A. Collin, A. Bordas, K. Verbeke, G. Vansant, E. Decuypere, and J. Buyse. 2007. Further investigations on the role of diet-induced thermogenesis in the regulation of feed intake in chickens: Comparison of adult cockerels of lines selected for high or low residual feed intake. Poult. Sci. 86:1960–1971.

Vogtmann, H., P. Frirter, and A. L. Prabuck. 1975. A new method of determining metabolizability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 16: 531–534.

Wenk, C. 1997. Energy metabolism of farm animals. Pages 265–267 in Proceedings of the 14th Symposium on Energy Metabolism. K. J McCracken, E.F. Unsworth, and A.R.G. Wylie. Newcastle, Co. Down, Northern Ireland. University Press, Cambridge.

Zuidhof, M. J. 2019. A review of dietary metabolizable and net energy: Uncoupling heat production and retained energy. J. Appl. Poult. Res. 28:231–241.

Zuidhof, M. J., M. V. Fedorak, C. C. Kirchen, E. H. M. Lou, C. A. Ouellette, and I. I. Wenger. 2016. System and method for feeding animals. PrecisionZX, Inc., assignee. United States Patent Application No. 15/283,125.

Zuidhof, M. J., M. V. Fedorak, C. A. Ouellette, and I. I. Wenger. 2017. Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity. Poult. Sci. 96:2254-2263.

Zuidhof, M. J., D. E. Holm, R. A. Renema, M. A. Jalal, and F. E. Robinson. 2015. Effects of broiler breeder management on pullet body weight and carcass uniformity. Poult. Sci. 94:1389–1397.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. 2014. Robinson. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult. Sci. 93: 2970–2982.

3.8 Tables

Table 3-1. Composition and calculated analysis of broiler breeder pullet grower diet provided from 10 to 23 wk of age.

Ingredient	g/kg
Corn	250
Wheat	470
Soybean meal	50
Oats	97.6
Canola meal	90
Ground limestone	10.8
Dicalcium phosphate	15.3
Choline chloride premix	5.0
Vitamin premix[1]	2.5
Mineral premix[2]	2.5
NaCl	4.5
D, L-methionine	0.9
L-lysine	0.4
Enzyme[3]	0.5
Total:	1,000
Analyzed composition, as fed basis	
AME (kcal/kg)	2,744
CP (g/kg)[4]	158
Calculated composition, as fed basis	
AME (kcal/kg)	2,900
CP (g/kg)	155
Calcium (g/kg)	9
Non-phytate phosphorous (g/kg)	4.2
Available lysine (g/kg)	6.8
Available methionine (g/kg)	3.7
Available methionine + cysteine (g/kg)	6.5

[1] Premix provided per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; cholecalciferol, 4,000 IU; Vitamin E (DL- α-tocopheryl acetate), 50.0 IU; vitamin K, 4.00 mg; pantothenic acid, 15.0 mg; riboflavin, 10.0 mg; folacin, 2.00 mg; niacin, 65 mg; thiamine, 4.00 mg; pyridoxine, 5.00 mg; vitamin B12, 0.02 mg; biotin, 0.20 mg

[2] Premix provided per kilogram of diet: iodine, 1.65 mg; Mn, 120 mg; Cu, 20.0 mg; Zn, 100 mg; Se, 0.30 mg; Fe, 80.0 mg

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

[4] Analyzed N using Leco TruMac (Leco Corporation, St. Joseph, Michigan, USA)

Table 3-2. Metabolizable energy intake (MEI) of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age.

	Treatment x Age Effect		Age Effect
	PF	CON	Overall
Age (wk)	kcal/d		
10 to 11	174[a]	122[b]	148[g]
11 to 12	156	127	141[g]
12 to 13	157	147	152[g]
13 to 14	128	150	139[g]
14 to 15	122[b]	159[a]	140[g]
15 to 16	157	153	155[g]
16 to 17	142[b]	221[a]	182[f]
17 to 18	210	182	196[f]
18 to 19	166[b]	277[a]	222[e]
19 to 20	174[b]	213[a]	193[f]
20 to 21	236	257	246[d]
21 to 22	274	287	280[c]
22 to 23	166[b]	227[a]	196[f]
Treatment Effect	174[s]	194[r]	-
Source of variation	DF	SEM	P-value
Treatment	1	3.23	< 0.001
Age	12	8.23	< 0.001
Treatment x Age	12	11.64	< 0.001

[a-b] means within row within treatment x age effect with no common superscript differ ($P < 0.05$)
[c-g] means within age effect with no common superscript differ ($P < 0.05$)
[r-s] means within treatment effect with no common superscript differ ($P < 0.05$)

Table 3-3. Body weight of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age.

	Treatment x Age Effect		Age Effect
	PF	CON	Overall
Age (wk)	g		
10	1,033[a]	977[b]	1,005[p]
11	1,169[a]	1,061[b]	1,115[o]
12	1,255[a]	1,149[b]	1,202[n]
13	1,308[a]	1,221[b]	1,265[m]
14	1,354[a]	1,300[b]	1,327[l]
15	1,415[a]	1,365[b]	1,390[k]
16	1,482[a]	1,433[b]	1,458[j]
17	1,584[a]	1,538[b]	1,561[i]
18	1,715[a]	1,645[b]	1,680[h]
19	1,870[a]	1,751[b]	1,811[g]
20	2,048[a]	1,885[b]	1,966[f]
21	2,200[a]	2,039[b]	2,120[e]
22	2,391[a]	2,226[b]	2,308[d]
23	2,610[a]	2,424[b]	2,517[c]
Treatment Effect	1,674[r]	1,572[s]	-
Source of variation	DF	SEM	P-value
Treatment	1	5.3	< 0.001
Age	12	10.1	< 0.001
Treatment x Age	12	14.8	< 0.001

[a-b] means within row within treatment x age effect with no common superscript differ ($P < 0.05$)
[c-o] means within age effect with no common superscript differ ($P < 0.05$)
[r-s] means within treatment effect with no common superscript differ ($P < 0.05$)

Table 3-4. Average daily gain of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age.

	Treatment x Age Effect		Age Effect
	PF	CON	
Age (wk)	g/d		
10 to 11	11.8	9.7	10.7[def]
11 to 12	11.6	15.1	13.4[bcd]
12 to 13	2.5	6.8	4.6[f]
13 to 14	9.9	16.2	13.0[cde]
14 to 15	8.8	3.6	6.2[ef]
15 to 16	9.9	15.7	12.8[cde]
16 to 17	18.3	15.2	16.7[abcd]
17 to 18	18.5	15.2	16.8[abcd]
18 to 19	21.8	15.1	18.5[abc]
19 to 20	21.3	18.8	20.0[ab]
20 to 21	20.6	21.7	21.2[a]
21 to 22	21.6	22.5	22.0[a]
22 to 23	23.3	23.0	23.1[a]
Treatment Effect	15.4	15.3	-
Source of variation	DF	SEM	P-value
Treatment	1	0.96	0.94
Age	12	2.45	< 0.001
Treatment x Age	12	3.47	0.72

[a-f] means within age effect with no common superscript differ ($P < 0.05$)

Table 3-5. Daily total heat production (HP), residual feed intake (RFI), residual daily total heat production (RHP), and proportion of expected ME intake (MEI) partitioned to HP and growth by breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age.

	PF	CON	SEM	P-value
Total HP[1] (kcal/kg$^{0.68}$)	111	129	0.54	< 0.001
RFI[2] (kcal/d)	-3.8	3.7	2.84	0.064
RHP[3] (kcal/kg^{b})	-0.12	0.12	0.47	0.73
Total HP (% of MEI)	86.9	89.0	0.61	0.020
Growth (% of MEI)	13.1	11.0	0.62	0.020

[1] Calculated using a nonlinear mixed model: $MEI = (120 + u) \times BW^{0.68} + 1.52 \times ADG + \varepsilon$, where $u \sim N(0,\sigma^2_u)$; ADG = average daily gain

[2] Calculated using residuals of the nonlinear mixed model:

RFI = observed MEI - predicted MEI

$MEI = (120 + u) \times BW^{0.68} + 1.52 \times ADG + \varepsilon$

[3] Calculated as the residual of the regression between a + u and MEI for each pen:

$a + u = 1.56 + 0.87\ MEI + \varepsilon$

where a + u = predicted total HP; ε = RHP

Table 3-6. Proportional breast muscle and abdominal fat pad weights of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age.

	Breast muscle						Fat pad					
	Treatment x Age Effect				Age Effect		Treatment x Age Effect				Age Effect	
Age (wk)	PF	SEM	CON	SEM	Overall	SEM	PF	SEM	CON	SEM	Overall	SEM
	% of live BW											
16	16.3	0.60	16.1	0.45	16.2^{y}	0.44	0.2	0.10	0.2	0.10	0.2^{y}	0.10
23	19.7	0.48	19.9	0.41	19.8^{z}	0.41	1.2^{b}	0.17	1.7^{a}	0.14	1.4^{z}	0.13
Treatment Effect	18.0	0.46	18.0	0.39	-		0.7^{s}	0.12	0.9^{r}	0.11	-	
Source of variation	DF		P-value				DF		P-value			
Treatment	1		0.92				1		0.002			
Age	1		< 0.001				1		< 0.001			
Treatment x Age	1		0.49				1		0.015			

$^{a-b}$ means within variable within row for treatment x age effect with no common superscript differ ($P < 0.05$)

$^{y-z}$ means within variable within age effect with no common superscript differ ($P < 0.05$)

$^{r-s}$ means within variable within treatment effect with no common superscript differ ($P < 0.05$)

Table 3-7. Cumulative feed conversion ratio of breeder pullets on precision feeding (PF) and conventional skip-a-day (CON) feeding treatments from 10 to 23 wk of age.

	Treatment x Age Effect		Age Effect
	PF	CON	
Age (wk)	g feed:g gain		
10 to 11	2.273[b]	3.510[a]	2.982[h]
10 to 12	3.063[b]	4.033[a]	3.548[g]
10 to 13	3.453	3.850	3.651[g]
10 to 14	4.192	4.494	4.343[ef]
10 to 15	4.236	4.306	4.271[f]
10 to 16	4.512	4.901	4.707[cd]
10 to 17	4.600	5.011	4.806[c]
10 to 18	4.538	4.842	4.690[cd]
10 to 19	4.325[b]	5.176[a]	4.750[cd]
10 to 20	4.096[b]	5.107[a]	4.601[cde]
10 to 21	4.094[b]	5.087[a]	4.591[cde]
10 to 22	4.189[b]	5.047[a]	4.618[cde]
10 to 23	4.057[b]	4.888[a]	4.472[def]
Treatment Effect	3.972[s]	4.635[r]	-
Source of variation	DF	SEM	P-value
Treatment	1	0.04	< 0.001
Age	12	0.11	< 0.001
Treatment x Age	12	0.16	0.002

[a-b] means within row for treatment x age effect with no common superscript differ ($P < 0.05$)
[c-h] means within age effect with no common superscript differ ($P < 0.05$)
[r-s] means within treatment effect with no common superscript differ ($P < 0.05$)

3.9 Figures

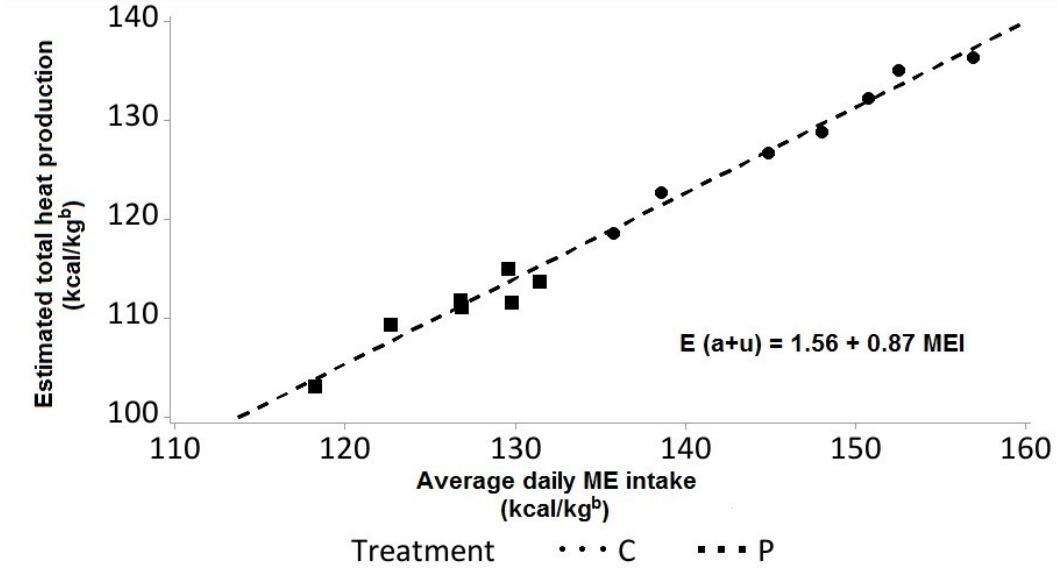

Figure 3-1. Regression of pen-specific total heat production [E(a + u)] versus ME intake ($R^2 = 0.99$; $P < 0.001$). The coefficient b for metabolic BW (kg^b) was independently estimated in the first stage (nonlinear) regression (b = 0.68). Average daily ME intake (MEI; $kcal/kg^b$) from 10 to 23 wk of age was calculated for each pen for precision feeding (P) and conventional skip-a-day feeding (C) treatments. Intercept and slope SEM were 3.96 and 0.028, respectively.

4 Chapter 4: Energy Partitioning by Broiler Breeder Hens in Conventional Daily Restricted Feeding and Precision Feeding Systems

4.1 Abstract

An empirical linear mixed model was derived to describe metabolizable energy (**ME**) partitioning in broiler breeder hens. Its coefficients described ME used for total heat production (**HP**), growth (**ADG**), and egg mass (**EM**). A total of 480 Ross 308 hens were randomly and equally assigned to two treatments: precision feeding (**PF**) and conventional daily restricted feeding (**CON**) from 23 to 34 wk of age. The PF system allowed birds to enter feeding stations voluntarily at any time, weighed them, and provided access to feed for 60 s if their body weight (**BW**) was less than the breeder-recommended target BW. The CON birds were fed daily each morning. Energetic efficiency of hens was evaluated using residual feed intake (**RFI**), defined as the difference between observed and predicted ME intake (**MEI**). The energy partitioning model predicted ($P < 0.05$) $MEI = A \times BW^{0.67} + 1.75 \times ADG + 0.75 \times EM + \varepsilon$. The coefficient A, a vector of age-specific HP, was 142 $kcal/kg^{0.67}/d$; the energy requirement for growth and EM was 1.75 and 0.75 kcal/g, respectively. For the CON and the PF hens, respectively, MEI was 366 and 354 kcal/d ($P = 0.006$); RFI was -5.9 and 6.7 kcal/d ($P = 0.009$); HP% was 85.5 and 87.7 ($P < 0.001$); hen-day egg production (**HDEP**) was 65.5 and 55.2% ($P < 0.001$). Although the CON hens had higher MEI, the model predicted lower HP%, thus CON hens had more nutrients available for egg production, increased egg production and were more energetically efficient than the PF hens. The decreased egg production by the PF hens was likely due to these hens receiving production-

related feed increases after an egg was laid. However, feed allocation increases for the CON hens resulted in increasing MEI for all CON hens at the same time. Therefore, the PF hens had lower ME intake and lower HDEP than the CON hens.

Key words: Caloric restriction, precision livestock farming, maintenance requirements, body composition, meat-type chicken

4.2 Introduction

Broiler body weight (**BW**) at 56 d of age has increased by over 450% due to intensive genetic selection from 1957 to 2005 (Zuidhof et al., 2014) whereas the target BW for broiler breeders has remained almost constant (Renema et al., 2007b). Thus, the gap between the growth potential of broilers and broiler breeder target BW is increasing, and consequently the degree of feed restriction in broiler breeders has become more severe and modern broiler breeders have reduced fat deposition (Renema et al., 2007b; Zuidhof, 2018). It was reported that modern broiler breeders had abdominal fat pad weights of only 0.44% of BW at 20 wk of age (van Emous, 2015) compared with 2.8% in 1989 (Bowmaker and Gous, 1989) and 1993 (Fattori et al., 1993). Zuidhof et al. (2014) demonstrated that in 2005, broiler chickens, the offspring of broiler breeders, had less body fat and more body protein compared with broiler chickens in 1957 and 1978. Broiler breeder hens from a line unselected since 2000 had abdominal fat pad that weighed 2.65% of BW, and a delayed onset of sexual maturation of 19.2 d compared with hens from a line unselected since 1980 that had abdominal fat pad of 5.38% of BW (Eitan et al., 2014). These results suggest that modern broiler breeders have reduced body fat due to more severe feed restriction and this can reduce egg production during

the laying period. A relaxation in the severity of feed restriction can increase fat pad deposition and egg production in broiler breeders (Zuidhof, 2018).

de Beer and Coon (2007) reported that broiler breeder hens fed on daily restricted program in rearing and laying period, produced more eggs than hens fed on skip-a-day feeding in rearing period and daily restricted feeding on laying period. The reason was probably due to more frequent availability of dietary nutrients in a daily-restricted feeding program, since deposition and mobilization of nutrients are not completely efficient processes (de Beer and Coon, 2007). On the other hand, broiler breeder hens fed on a 5-2 feeding program (feeding 5 consecutive days and off for 2 non-consecutive days) had larger eggs than those fed according to a daily-restricted feeding program (de Beer and Coon, 2007). Repeated fasting and refeeding cycles in 5-2 birds caused conversion of dietary carbohydrates to lipid and deposition of lipid in the carcass (de Beer and Coon, 2007). Some of the lipid is mobilized during the fasting period to meet energy requirements and some is stored in the carcass (de Beer and Coon, 2007). There is probably an increase in hepatic lipogenesis and the deposition of more carcass fat which may result in larger eggs in feeding programs that include off-feed days (de Beer and Coon, 2007).

Metabolizable energy intake is partitioned to maintenance, growth, and egg production in broiler breeders (Sakomura, 2004). The maintenance requirement (ME_m) includes energy required for basal metabolism, thermal regulation, immune responses and activity (NRC, 1981); since the ME_m is the sum of all unretained ME, it is the same as total HP (Zuidhof, 2019). Predicting total HP will help nutritionists

to precisely match energy supply (available energy in feed) to energy needed for maintenance, growth, and production (Zuidhof, 2019). Empirical ME intake models quantify the amount of dietary ME partitioned to maintenance (HP), ME retained in the body (average daily gain, **ADG**), and egg mass produced by broiler breeder hens (Romero et al., 2009b; Pishnamazi et al., 2015). Residual feed intake (**RFI**), the difference between observed and model-predicted ME intake can be used to identify efficient animals and differences due to management or feeding system (Romero et al., 2009a; Luiting and Urff, 1991).

To increase efficiency, ensure equitable feed distribution for every individual bird, and increase consistency in supply of nutrients to increase egg production, new feed restriction methods or new feeding technologies may be needed for modern broiler breeders. A novel precision feeding (**PF**) system was developed at the University of Alberta to provide the right amount of feed to each bird, increase BW uniformity, and increase efficiency through increasing consistency in nutrient supply because it provides several small meals in a day for an individual bird (Zuidhof et al., 2016, 2017). The PF system individually weighs broiler breeders and makes decisions in real time about whether or not to feed them after comparing their actual BW with their target BW.

The first objective for the current experiment was to develop a ME intake model whose coefficients quantified the amount of ME partitioned to total HP, ADG, and egg mass of broiler breeder hens. The second objective was to use this model to compare energetic efficiency between a conventional daily restricted feeding program and the PF system. The third objective was to evaluate the effect

of a conventional daily restricted feeding program and the precision feeding system on egg production, egg mass, total HP, ADG, carcass composition and age at 50% production as an indicator of sexual maturation. It was hypothesized that precision-fed broiler breeders hens would be more energetically efficient because of a higher feeding frequency and would partition more energy toward egg production compared with the hens in the conventional daily restricted feeding program.

4.3 Materials and Methods

4.3.1 Experimental Design

The current study was approved by Animal Care and Use Committee Livestock at the University of Alberta and followed the Canadian Council on Animal Care guidelines (CCAC, 2009). Four hundred and eighty Ross 308 broiler breeder hens were randomly and equally assigned to two treatments (8 pens of 30 hens in each treatment): 1) precision feeding system (**PF**) and 2) conventional daily restricted feeding (**CON**) in a randomized complete block design. Eight environmentally controlled chambers (blocks) were used, and each block contained one replicate pen of each treatment. Prior to the current experiment, in the rearing period from 10 to 23 wk of age, PF hens were fed using the PF system, and CON hens were fed using a skip-a-day feeding program (Hadinia et al., 2018). The PF and the CON hens were reared to achieve the breeder-recommended Ross 308 BW target (Aviagen, 2011), and the interpolated BW target was updated hourly in the PF treatment by the PF system. In the CON treatment, pullets were fed each morning and feed allocation decisions for the CON treatment were made based on weekly BW measurements, to maintain breeder recommended BW targets. The BW

CV for the CON and the PF pullets at 23 wk of age was 13% ± 0.11 and 8% ± 0.11 respectively ($P < 0.001$, data not shown). For the laying period, broiler breeder pullets were randomly reassigned to new pens within the same treatment.

4.3.2 Precision and Conventional Feeding Treatments

The PF hens in each pen (n = 30) were fed using one feeding station per pen, the design and function of which are fully described elsewhere (Zuidhof et al., 2016, 2017). Briefly, each PF hen was identified by a unique radio frequency identification (**RFID**) tag and weighed by a built-in platform scale when she entered the PF station. If her BW was equal to or greater than the target BW, the PF hen was gently ejected by the station. However, if her BW was lower than the target BW, access to approximately 25 g of feed was provided for one minute, after which the hen was ejected from the station. Before and after feeding, the feeder that was mounted on a load cell was weighed; feed intake for each feeding bout was calculated as the initial minus the final feed weight. After weighing the feed at the end of each bout, the feeder was topped up to provide approximately 25 g of feed for the next feeding bout. For each visit, RFID, BW, and initial and final feed weight data were written to a database with a date and time stamp. Monochromatic green LED lights (525 nm wavelength) mounted above the feeder provided a light intensity of 1.9 lux at the feeder position. The green wavelength was strategically chosen to help pullets to see well enough to enter the feeding stations and eat during the scotophase without stimulating hypothalamic photoreceptors (Rodriguez, 2017). Thus, PF hens could access feed 24 hours per day and consume several small meals over a day, whereas the CON hens received a single large meal per day. Each

week, all hens in both treatments were individually weighed manually. The ADG was calculated for each experimental unit (pen).

4.3.3 Management

Each experimental pen contained pine shavings litter at a depth of approximately 5 cm. Two suspended nipple drinkers (4.4 birds per nipple) provided water ad libitum throughout the experiment. The stocking density was 7.4 birds/m^2. There were three hanging tube feeders in each CON pen, providing 10 cm of feeder space per hen. The feeder in each PF station was 4.8 cm wide, and only one bird at a time was allowed access to feed. Temperature was 21 ± 0.36°C during the entire experimental period. Photoperiod was gradually increased by one hour per week from 8L:16D to 14L:10D at 23 wk of age, and was accompanied by an increase in light intensity from 10 to 30 lux in one step. A single broiler breeder layer diet (Table 4-1) was formulated according to the Ross 308 recommendations (Aviagen, 2013) and was provided in pellet form to both treatments for the duration of the study.

4.3.4 Measurement of AME

Two percent of an acid-insoluble ash marker (Celite 281, Lompoc, CA) was added to the diet for 4 consecutive d beginning at each of 23, 28 and 32 wk of age to allow calculation of AME content of the diet. In total, 16 hens per treatment (2 hens per pen) were randomly selected after 4 days of consumption of the celite containing feed, and euthanized by cervical dislocation. Ileal digesta samples were collected from Meckel's diverticulum to the ileal-cecal-colon junction of the intestinal tract. Digesta samples were pooled within each pen and frozen at -20°C

until further analysis. Diet and ileal digesta samples were oven dried at 60°C for 48 h and then ground. Samples were digested with 4N HCl and then the residues were ashed at 500 °C (Vogtmann et al., 1975). Using bomb calorimetry, gross energy (**GE**) of feed and digesta samples were measured. The AME values were calculated as described by Scott and Boldaji (1997):

$$\mathrm{AME} = \mathrm{GE}_{\mathrm{diet}} - \mathrm{GE}_{\mathrm{digesta}} \times \frac{\mathrm{Marker}_{\mathrm{diet}}}{\mathrm{Marker}_{\mathrm{digesta}}}$$

where GE = gross energy (kcal/kg of sample); and Marker = concentration of acid insoluble ash in sample. Because the diet did not contain the acid insoluble ash marker at all times a 2% percent correction factor was applied to the dietary AME values. The AME values were not corrected for nitrogen retention and were expressed on an as-fed basis. Nitrogen content of feed was determined by the combustion method using a Leco TruMac N machine (Leco Corporation, St. Joseph, Michigan, USA) and dietary CP was estimated using a factor of 6.25 (Hossain et al., 2012; Mutucumarana et al., 2015).

4.3.5 Carcass Traits

4.3.5.1 Breast Muscle and Fat Pad

For carcass characteristics, 42 birds per treatment were euthanized at each of 23, 28, 32 and 34 wk of age, and the weights of breast muscle (P. major + P. minor) and abdominal fat pad were recorded. The weights of fat pad and breast muscle were reported as a percentage of live BW.

4.3.5.2 Oviduct and Ovarian Morphology

At each dissection age, the ovarian stroma, and oviduct were weighed and expressed as a percentage of BW. Each large yellow follicle (**LYF**, greater than 10 mm) was individually weighed at 34 wk of age and the number of hierarchical LYF was counted.

4.3.6 Age at 50% Production

Eggs were collected daily and average egg weight was determined for each pen. The number of normal eggs was calculated by deducting the number of abnormal eggs (double yolks, deformed and eggs with shell problems such as soft shelled eggs or shell-less eggs) from the number of total eggs. Hen-day egg production for each pen was calculated by dividing the number of normal eggs produced per day by the number of hens alive on that day. The egg mass (g/d) per hen for each pen was calculated as laying percentage multiplied by the average egg weight. To evaluate sexual maturation (from photostimulation to 50% egg production), age at 50% production was estimated for the CON and the PF treatments. Age at 50% production (parameter μ in the following model) was estimated using a nonlinear model as described by Renema et al. (2007a).

$$\text{Hen} - \text{day egg production} \times 1 + \exp^{-\frac{\pi}{3\sigma} \times (\text{age} - \mu)} = 100 \qquad \text{[Equation 1]}$$

where Hen-day egg production was calculated for each treatment per d (%); π to 4 decimal points was 3.1416; σ was the SD in age at 50% production; age was age of hens in d; μ was the average age (d) at 50% production. The parameters σ and μ were estimated directly using the NLIN procedure of SAS (SAS 9.1, SAS Institute

Inc., Cary, NC). Equation 1 was predicted under the assumption that sexual maturation follows a normal distribution (Yang et al., 1989).

4.3.7 Metabolizable Energy Partitioning Model

A linear mixed model, Equation 2, was used to derive ME requirements for total HP, ADG and egg mass of broiler breeder hens from 23 to 34 wk of age. The model was estimated with the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) and pen was a random effect.

$$\text{Observed MEI} = \text{Predicted MEI} + \varepsilon$$

$$\text{Predicted MEI} = A \times BW^{0.67} + c \times ADG + d \times EM$$

$$\text{Observed MEI} = A \times BW^{0.67} + c \times ADG + d \times EM + \varepsilon \quad \text{[Equation 2]}$$

where MEI was ME intake (kcal/d); observed MEI for each pen was calculated weekly by multiplying the energy of the diet (kcal/kg) by observed feed intake (kg). A was a vector of estimated age-specific (weekly) total HP coefficients (kcal/kg$^{0.67}$/d); BW was the average BW (kg) of each experimental unit during the week in which total HP was estimated; $BW^{0.67}$ was metabolic BW; c was the estimated coefficient of ADG (g/d), which defined the ME cost for each g of BW gain (**ME_g**, kcal/g); EM was egg mass and d was the estimated coefficient of egg mass (g/d), which defined the ME cost for each g of egg mass (**ME_e**, kcal/g); and ε was the residual or unexplained error (RFI) and was used to evaluate energetic efficiency.

4.3.8 Partitioning of ME Intake to Growth, Total HP, and Egg Mass

To partition ME intake to total HP, ADG, and egg mass in broiler breeder hens from 23 to 34 wk of age, the percentage of total HP to observed ME intake

(Total HP%), the percentage of ADG to observed ME intake (Growth%), and the percentage of egg mass (**EM**) to observed ME intake (Egg mass%), respectively were calculated weekly:

$$\text{Total HP\%} = \left[\frac{\text{A} \times \text{Metabolic BW}}{\text{Observed ME intake}}\right] \times 100$$

$$\text{Growth\%} = \left[\frac{\text{c} \times \text{ADG}}{\text{Observed ME intake}}\right] \times 100$$

$$\text{Egg mass\%} = \left[\frac{\text{d} \times \text{EM}}{\text{Observed ME intake}}\right] \times 100$$

4.3.9 Regression Analysis between ME Intake and Weekly Total HP

The relationship between total HP and ME intake from 23 to 34 wk of age was estimated by a linear regression, modified from Romero et al. (2009b) such that ME intake was expressed per unit of metabolic BW:

$$A = \text{intercept} + \text{slope} \times \text{MEI} + \varepsilon$$

where A was the total HP coefficient estimated for each pen (kcal/kg$^{0.67}$); MEI was ME intake (kcal/kg$^{0.67}$); slope (kcal/kg$^{0.67}$/kcal/kg$^{0.67}$) was the coefficient defining the linear rate of change of weekly total HP with respect to ME intake; and ε was the error term. Since the units for the slope coefficient cancel, the slope can be directly interpreted as the proportion of dietary energy lost by the hens as heat (Figure 4-1).

4.3.10 Egg Composition

In the current study, egg composition was not measured. However, to understand whether the result of the estimation for the coefficient d (ME_e = 0.75 kcal/g) in Equation 2 was biologically feasible, a set of calculations and estimations

were simply carried out using Excel (Microsoft, 2010). It was assumed that a 55 g egg consists of 10% eggshell (0.08 kcal/g), 60% albumen (0.47 kcal/g) and 30% of yolk (3.01 kcal/g; Abeyrathne et al., 2013; Radu-Rusu et al., 2012; Gilbert, 1971; McLeod et al., 2014). Weight and energy content of shell, albumen, and yolk were calculated for a 55 g egg:

Yolk energy = 30% x 55 g x 3.01 kcal/g = 49.7 kcal

Albumen energy = 60% x 55 g x 0.47 kcal/g = 15.5 kcal

Shell energy = 10% x 55 g x 0.08 kcal/g= 0.44 kcal

ME_e = 1.19 kcal/g for a 55 g egg was calculated by dividing the total energy content of the egg (65.6 kcal) by the weight of the egg (55 g). Next, using Excel Solver Add-in and ME_e = 1.19 kcal/g, yolk weight (6.91 g) and albumen weight (42.6 g) for ME_e = 0.75 kcal/g at the 55 g egg were estimated. Lastly, yolk and albumen percentage in broiler breeder hens from 24 to 34 wk of age were calculated:

$$\text{Yolk\%} = \left[\frac{\text{Yolk Weight for ME}_\text{e} = 0.75 \text{ at 55 g Egg}}{\text{Average Egg Weight in the Current Study}}\right] \times 100$$

$$\text{Albumen\%} = \left[\frac{\text{Albumen Weight for ME}_\text{e} = 0.75 \text{ at 55 g Egg}}{\text{Average Egg Weight in the Current Study}}\right] \times 100$$

4.3.11 Statistical Analysis

4.3.11.1 Analysis of Variance

Metabolizable energy intake, BW, ADG, egg weight, ovary and oviduct weights, and total HP ($A \times BW^{0.67}$), were evaluated as a 2–way ANOVA using MIXED procedure in SAS where age and treatment were considered as source of variation, and pen was a random effect. Egg mass, total HP%, growth%, egg mass%, RFI, and LYF weight were analyzed using the MIXED procedure as a 1–

way ANOVA, with feeding treatment as the main effect. Least significant difference test was applied to multiple mean comparisons. The PDIFF option of the LSMEANS statement used to estimate pairwise differences between means and least significant difference test was applied to multiple mean comparisons. Differences between means were reported as significant where $P \leq 0.05$. Trends were reported where $0.05 < P < 0.1$.

4.4 Results and Discussion

4.4.1 Metabolizable Energy Intake, Body Weight, and Average Daily Gain

The CON hens had 3% higher ME intake (366 kcal/d) compared with the PF hens (354 kcal/d) from 23 to 34 wk of age (Table 4-2). Metabolizable energy intake of CON hens was higher than PF hens from 23 to 28 wk of age, after which the ME intake of PF hens was higher than the CON hens. After 25 wk of age, CON hens were heavier than PF hens (Table 4-2) and after 27 wk of age CON hens were above the breeder-recommended target BW. The ADG of CON hens was higher than PF hens from 23 to 28 wk of age, after which ADG did not differ between treatments (Table 4-2). The feeding frequency and the meal size for the PF hens was 11.5 ± 0.26 meals per day, and 11.3 ± 0.14 g/meal respectively. In contrast, the feeding frequency for the CON hens was 1 meal per day since they were fed once on daily basis and their meal size was 135 ± 1.3 g/d on average from 23 to 34 wk of age.

The lower ME intake for the PF hens during the first 5 wk of the experiment than for the CON hens could have been due to several factors. First, the CON hens were weighed only once per week and the feed allocation decisions were made

weekly, considering their BW and desired rate of gain for the subsequent week. The BW of CON hens at the start of the laying period was lower than the PF hens (2,477 vs. 2,602 g respectively), thus the feed allocation for the CON hens was increased compared with PF hens which resulted in their higher ME intake. Because the feed allocation decisions for the CON hens were made much less frequently, they were more prone to error than the real-time feed allocation decisions for the PF hens. If the CON hens reached the target BW earlier than expected or delayed their achievement to the target BW, there was no chance of revising the feed allocation until the next feed allocation decision since they were weighed only once per week. Second, the feed allocation decisions for the PF treatment were based on the individual BW of each hen. If, for example, a PF hen laid a 50 g egg, her BW would be reduced by 50 g. Since she would then be lower than the target BW, she would continue to receive feed until her BW matched the target BW. This would effectively increase her feed allocation on any day she laid an egg. Conversely, feed increases for the CON treatment allowed for all hens to increase feed intake simultaneously. Therefore, a PF hen that did not lay an egg did not receive the same feed increase as a PF hen that did lay an egg. The current PF protocol provided feed increases after an egg was laid in each individual hen, but did not provide feed increases before the onset of egg production in the same manner as the conventional feeding protocol. Therefore, the PF hens had lower ME intake than the CON hens from 23 to 28 wk of age. Lower ADG of PF hens compared with CON hens from 23 to 28 wk of age was due to their lower ME intake from 23 to 28 wk of age. The CON hens had lower ME intake than the PF hens from 29 to 34 wk of age. The

reason was due to decreasing the feed allocations to match the BW with the target BW because the BW of the CON hens exceeded the target BW after 27 wk of age. However, the CON hens still had higher BW than the PF hens until wk 34. Obviously, feed allocation decisions are difficult and this challenge for the research team underscores this difficulty. Precision-fed broiler breeder hens compared with conventionally daily fed grandparent had lower average daily feed intake from 22 to 52 wk of age and it was hypothesized that feed restriction should be relaxed for precision-fed broiler breeders by increasing the target BW (Zuidhof, 2018).

4.4.2 Carcass Traits

Breast muscle as a percentage of BW did not differ between the CON and the PF hens at any age (Table 4-3). At 23 and 28 wk of age, hens in the CON treatment had increased fat pad compared with hens in the PF treatment. However, there was no difference in fat pad percentage after 28 wk of age. The CON hens had higher ME intake than the PF hens from 23 to 28 wk and this explained the higher fat pad percentage in the CON hens during that period. Less frequent feeding (skip-a-day) in compared with more frequent feeding (daily restricted) increased expression of genes involved in hepatic lipogenesis 12 and 24 h after feeding (de Beer et al., 2007). The CON feeding program used in the current study that involved less frequent feeding probably provided just such a scenario. The less frequency of feeding in the CON hens would likely have increased hepatic lipogenesis compared with the PF hens. In addition, the higher ME intake of the CON hens than the PF hens from 23 to 28 wk of age would likely have provided sufficient energy for the CON hens to store some energy in the abdominal fat pad. Abdominal fat pad weight

is linked directly to total body fat content in avian species thus it is a reliable indicator for judging total body fat content (Thomas et al., 1983; Becker et al., 1979). In the current study, the CON hens deposited more energy in the fat pad thus they had more energy available for reproductive development and egg production.

4.4.3 Sexual Maturation and Egg Production

Overall hen-day egg production from 24 to 34 wk of age was higher for the CON hens (65.5%) compared with the PF hens (55.2%; Table 4-4). The peak of hen-day egg production for the CON treatment was 87.6% from 32 to 33 wk of age, compared with 82.0% for the PF treatment from 33 to 34 wk of age. The PF hens likely did not peak by 34 wk of age. Age at 50% production was estimated to be 192.7 d ± 0.56 for the CON hens, 8.5 d earlier than the PF hens (201.2 d ± 0.75; $P < 0.05$).

From 23 to 28 wk of age, the CON hens had 14.8% higher ME intake and at 23 and 28 wk of age, the CON hens had 29.4 and 20.8% higher fat pad weights respectively than the PF hens. Moreover, the CON hens were 7% heavier than the PF hens from 26 to 34 wk of age. Thus, the CON hens had more energy available in the body and they increased hen-day egg production compared with the PF hens from 25 to 33 wk of age. Broiler breeder hens utilize body lipid reserves as an energy source for egg production (Nonis and Gous, 2012). Body fat content affects yolk synthesis because incorporation of lipids originating from diet, lipoproteins from liver, and release of lipids from adipose tissue contributes to yolk synthesis (Yang et al., 2013). Renema et al. (2007a) reported that hens with higher fat pad content reached 50% production 7 days earlier than hens with lower fat pad content

($P < 0.05$). The CON hens likely had more lipid resources available in the body, which may have advanced the age at 50% production and increased egg production relative to the PF hens. Age at 50% production was delayed in the PF hens because the feed allocation increases for the PF hens was provided after the hen had laid an egg. Thus, the PF hens had limited nutrients to form the egg unlike the CON hens, which received feed increases prior to laying the egg. The CON hens reached 50% production earlier, indicating the rate of pubertal growth was faster and advanced sexual maturation than the PF hens. The hypothalamic-pituitary-gonadal (**HPG**) axis controls sexual maturation (Bédécarrats et al., 2009). Advancing sexual maturation in the CON hens relative to the PF hens suggested that the higher ME intake and greater fat pad deposition could advance the activation of the HPG axis. Although the PF hens at the end of study (wk 34) attained approximately the target BW, they delayed sexual maturation and did not have increased egg production relative to CON hens. Precision-fed broiler breeder hens compared with conventionally fed grandparent hens had lower egg production from 22 to 52 wk of age and 1.2 times greater breast muscle weight at wk 22, however abdominal fat pad weight did not differ between the groups of hens at 22 wk (Zuidhof, 2018). van der Klein et al. (2018a) demonstrated that total egg production from 20 to 55 wk of age was lower for the hens in a standard breeder-recommended target BW (92.8) relative to the hens in a high BW treatment reaching the standard 21 wk BW at 18 wk (129.4). Moreover, fat pad weight at 55 wk of age respectively was lower for the hens in the standard treatment (1.6%) compared to the hens in the high treatment (2.2%; van der Klein et al., 2018b). Therefore, the target BW for the PF hens would

likely have to be increased before the laying period and also around the time of sexual maturation to provide a sharp increase in feed intake and help the PF birds to increase their ME intake, and body fat deposition to advance the activation of the HPG axis which likely leading to advance sexual maturation and higher production.

4.4.4 Egg Weight and Egg Mass

Egg weight did not differ between the CON and the PF treatments except at 31 wk of age. At 31 wk of age, the PF hens had 1.5 g greater egg weight than the CON hens (Table 4-4), likely due to their higher ME intake from 29 to 34 wk of age. The CON hens had higher egg mass (38.6 g/d ±0.69) than the PF hens (33.1 g/d ± 0.69) from 24 to 34 wk of age ($P < 0.001$). Hen-day egg production was higher for the CON hens than the PF hens from 25 to 33 wk of age. Romero et al. (2009b) reported that egg mass was 4.3 g lower in hens with low target BW (standard × 0.9) compared with hens with high target BW (standard × 1.1). This result and the result of the current study are consistent with the hypothesis that egg production was limited by ME intake for hens with lower BW which resulted in decreased the egg mass. Therefore, in the current study, the higher production levels resulted in higher egg mass for the CON hens than the PF hens.

4.4.5 Ovarian Morphology

The CON hens had higher relative ovary and stroma weights than the PF hens at 28 and 32 wk of age (Table 4-5). Moreover, the CON hens had a higher relative oviduct weight compared with the PF hens at 28 wk of age (Table 4-5). The greater weights of ovary and stroma and oviduct for the CON hens relative to the PF hens suggested a more advanced ovary development in the CON hens. This has

resulted in advancing sexual maturation for the CON hens, which is consistent with the age at 50% production for the CON hens being 8.5 d earlier than the PF hens. Weight and number of LYF (F1-F7) did not differ between the CON and the PF hens at 34 wk of age (Table 4-6). The average number of LYF was 6.03 at 34 wk of age. Similarly, Joseph et al. (2002) reported 6.16 LYF at 32 wk of age for Cobb 500 broiler breeder hens and Hocking (1987) reported 5.6 LYF in dwarf breeders at 30 wk of age. Although the growth potential for modern broiler breeders has increased during the last 30 years and feed restriction has become more severe (Renema et al., 2007b), the number of LYF does not appear to have changed.

4.4.6 Metabolizable Energy Partitioning

The ME partitioning model was ($P < 0.001$):

$$MEI = A \times BW^{0.67} + 1.75 \times ADG + 0.75 \times EM + \varepsilon \qquad \text{[Equation 3]}$$

4.4.6.1 The ME Requirement for BW Gain

The estimated ME_g was c = 1.75 kcal/g using the linear model, Equation 3. A value of 2.13 was reported for ME_g kcal using non-linear models in Ross 708 broiler breeder hens from 25 to 41 wk of age (Pishnamazi et al., 2015). Romero et al. (2009b) estimated 2.94 kcal/g for ME_g in Ross 708 broiler breeder hens using a non-linear model and the interaction of metabolic BW and ADG ($1.18BW^{0.60}ADG^{1.10}$) from 20 to 60 wk of age. It is possible that ME_g estimates might differ in part due to modeling methodology, but may also vary due to differences in strain, and composition of gain. To have an understanding of body compositions of broiler breeders using the estimated ME_g, a simple system of equations was used:

$$1.38\ X + 9.1\ Y = ME_g$$

$$X + Y = 1$$

Where 1.38 was the ME requirement per g of lean tissue (kcal/g; Claus and Weiler, 1994; Hadinia et al., 2018); X was the lean tissue as a proportion of total gain; 9.1 was the ME requirement per g of fat tissue (kcal/g; Johnston, 1970); Y was the fat tissue as a proportion of total gain (kcal/g); and ME_g was ME requirement per g of ADG (kcal/g). These formulas predicted broiler breeders in the study by Romero et al. (2009b, ME_g = 2.94 kcal/g), deposited 21 and 79% fat and lean tissues respectively. Moreover, these formulas predicted broiler breeders in the study by Pishnamazi et al. (2015, ME_g = 2.13 kcal/g), deposited 10 and 90% fat and lean tissues respectively. These formulas predicted broiler breeders in the current study (ME_g = 1.75 kcal/g) deposited 5 and 95% fat and lean tissues respectively. The coefficient c = 1.75 kcal/g estimated in the current experiment is close to the value of retained energy of lean tissue (1.38 kcal/g) and it is consistent with the observation that modern broiler breeders deposit primarily lean tissue under conventional feed restriction (Hadinia et al., 2018; van Emous, 2015). A greater ME_g is expected at higher BW because broiler breeders increase body fat with age (Sakomura, 2004). Because of continued selection for broiler traits, broiler breeders deposit less fat as a proportion of total gain and therefore ME_g is continually being reduced.

4.4.6.2 Total Heat Production

The CON and the PF hens partitioned ME intake differently from 23 to 34 wk of age. The CON and the PF hens, respectively, partitioned 85.5 and 87.7% of

their total ME intake toward maintenance, which eventually was lost as total HP (Table 4-7). The CON and the PF hens, respectively, partitioned 7.1 and 5.6% of their ME intake toward growth (Table 4-7). Additionally, the CON hens partitioned more ME intake into egg mass than the PF hens (7.4 vs. 6.7% respectively; Table 4-7). The age-specific total HP (A) was reduced from 156 kcal/$kg^{0.67}$/d at wk 23 to 123 kcal/$kg^{0.67}$/d at wk 28 (Table 8). Total HP (A × $BW^{0.67}$) was higher for the CON hens than the PF hens from 25 to 34 wk of age (Table 4-8).

The first priority of animals is to satisfy the maintenance energy requirement, after which energy can be partitioned to growth and egg production (Pishnamazi et al., 2015). The CON hens had higher ME intake from 23 to 28 wk of age, higher BW from 26 to 34 wk of age, and higher total HP (A × $BW^{0.67}$) from 25 to 34 wk of age. However, the model predicted that the CON hens would proportionally partition less energy toward maintaining their body (total HP%) and proportionally more energy toward growth (growth%) and egg mass (egg mass%) from 23 to 34 wk of age than the PF hens. Since the CON hens partitioned proportionally more energy toward egg mass, they increased hen-day egg production and eventually became more energetically efficient than the PF hens.

Physical activity accounted for about 22% of total HP in laying hens (Saiful et al., 2002), and about 20% in broiler breeders (Sakomura, 2004). In the current study, the activity levels of the hens were not estimated. However, there may have been differences in the level of activity, which we did not observe. It was possible that the CON hens had a higher level of activity from 25 to 34 wk of age because they had higher total HP during that period. It was suggested that ME_m (total HP)

was lower in fat animals than lean animals because fat tissue contributes little to HP compared with protein (Close, 1990). In the current experiment, the increased fat pad weight from 23 to 34 wk of age could be reason for the reduction in the age-specific total HP (A) from 156 kcal/$kg^{0.67}$ at wk 23 to 123 kcal/$kg^{0.67}$ at wk 28. Protein synthesis accounted for between 16 to 26% of total HP in pigs, and lipid synthesis accounted for between 1 to 3% of total HP in rats (Reeds et al., 1982). In poultry, there is lack of information about the proportion of total HP accounted for by protein and lipid synthesis. Although the CON hens had greater fat pad weight than the PF hens at 23 and 28 wk of age, the CON hens were allocated higher ME intake than the PF hens during that period because their BW was lower than the target BW until 27 wk of age. Increasing feed intake increases diet-induced thermogenesis (the energy expended to digest feed; Pishnamazi et al., 2008). Moreover, the CON hens had higher BW than PF hens from 26 to 34 wk of age, which resulted in their higher total HP from 25 to 34 wk of age. Larger broiler breeders have higher total HP than smaller broiler breeders (Leeson and Summers, 2000). Thus, diet-induced thermogenesis and BW likely contributed at least in part to the higher total HP ($A \times BW^{0.67}$) by the CON hens from 25 to 34 wk of age. Regression analysis between ME intake and weekly total HP defined the following equation ($P < 0.001$; Figure 4-1):

$$A = 5.7 + 0.91\ MEI$$

Thus, total HP (A) increased linearly with increasing ME intake (MEI) within the ranges reported for A (123 to 177 kcal/$BW^{0.67}$) and MEI (134 to 189 kcal/$BW^{0.67}$). Specifically, the model predicted that 91% of every additional kcal of ME

consumed within the reported intake range was lost as heat. Although the estimation of 91% was different from the estimations of 85.5 and 87.7% for the ME partitioned toward total HP in the CON and PF hens respectively, the values are close to each other. The differences between these values could be related to the differences in the methodology. Moreover, the estimate of 91% focused on the upper part of the intake range vs. the total observed ME intake.

4.4.6.3 The ME Requirement for Egg Mass

The ME_e in the current study was d = 0.75 kcal of ME per g of egg mass and the mean egg mass was 38.6 and 33.1 g/d for the CON and the PF treatments respectively from 24 to 34 wk of age. Cobb 500 broiler breeder hens needed 2.30 kcal ME for each g of egg mass and their average egg mass was 50.47 g/d from 32 to 42 wk of age (Reyes et al., 2012). These researchers reported a greater value for the ME_e compared with the estimated values in the current experiment. These researchers determined the ME_e from the energy content of eggs, in contrast with the empirical linear model used in the current study. It is possible that ME requirement estimates might differ in part due to modeling methodology, but may also vary due to differences in egg mass, and egg composition. Increasing egg mass increased ME_e (Pishnamazi et al., 2015) because there is an increased energy cost with increasing egg mass production (Chwalibog, 1992). Romero et al. (2009b) reported a positive correlation (r = 0.85) between egg mass production and yolk deposition. Reyes et al. (2012) reported each egg in their study contained 29.5% yolk and 61% albumen. In the current study, with ME_e = 0.75 kcal/g and the average egg weight of 57.5 g, each egg contained 12% yolk and 74% albumen and the ratio

of yolk:albumen was 0.16. Nangsuay et al. (2011) reported that yolk:albumen ratio varied between 0.38 to 0.44 at wk 29 and between 0.55 to 0.57 at wk 53 in Ross 308 broiler breeders. This indicated that the estimated value for the ME_e (0.75 kcal/g) using the empirical model was biologically infeasible. Moreover, the estimation of the ratio of yolk to albumen showed that the empirical ME intake model probably underestimated the ME_e. This is one of the main flaws of mathematical models that there is absence of a way to describe energy balance to incorporate energy intake-related changes in maintenance requirement (Romero et al., 2009b) and in production requirement. The predicted model, Equation 3, did not appear to work well and the major limitation for that was probably the relatively small number of experimental units (16 pens). Moreover, the small sample size for egg collections (10 wk) could be another limitation for the predicted model, which led to an inaccurate prediction by the model. If eggs were collected for a longer period, including after peak production, the model would have a larger sample size.

4.4.7 Energetic Efficiency

In the current study, the CON hens had lower RFI compared with the PF hens (-5.9 and 6.7 kcal/d respectively, Table 4-7); thus they were more energetically efficient than the PF hens. Several authors reported that animals having a lower maintenance requirement (total HP), had lower RFI and were more efficient compared with animals having a higher total HP (Luiting and Urff, 1991; Sharma et al., 2018; Swennen et al., 2007; Basarab et al., 2003). Adult cockerels genetically selected for high RFI (inefficient) had 40% higher ME intake and 31% higher diet-

induced thermogenesis (as a percentage of ME intake) than cockerels genetically selected for low RFI (efficient; Gabarrou et al., 1997).

The CON hens had higher ME intake from 23 to 28 wk of age, higher BW from 26 to 34 wk of age, and higher total HP ($A \times BW^{0.67}$) from 25 to 34 wk of age. However, the model predicted that the CON hens partitioned more ME intake toward ADG and egg mass and less ME intake toward total HP than the PF hens. Moreover, the greater ovary and stroma weight at 28 and 32 wk of age and the greater oviduct weight at 28 wk of age for the CON hens showed that they partitioned more energy toward developing reproductive tissues than the PF hens. In addition, the CON hens had higher abdominal fat pad than the PF hens at 23 and 28 wk of age, indicating that the CON hens were in a state of higher energy balance, and likely had more resources available for egg production. These results indicated that the CON hens partitioned ME intake more efficiently than the PF hens.

4.5 Conclusions

In conclusion, the CON hens had higher ME intake from 23 to 28 wk of age, higher BW from 26 to 34 wk of age, and higher total HP ($A \times BW^{0.67}$) from 25 to 34 wk of age than the PF hens. The feed allocation decisions for the PF treatment were based on the individual BW of each hen, and the increase in feed allocation was provided after the hen laid the egg, thus the PF hens had limited nutrients to form the initial egg. Conversely, feed increases for the CON treatment allowed for all hens to increase feed intake and had sufficient nutrients to form the eggs and increase the egg production. Thus, the PF hens had lower ME intake than the CON hens from 23 to 28 wk of age. Increased diet-induced thermogenesis and body size

likely contributed at least in part to the higher total HP by the CON hens. However, the model predicted that the CON hens compared with the PF hens would partition less energy toward total HP and more energy toward growth and egg mass and eventually they increased egg production. This resulted in the higher energetic efficiency of the CON hens than the PF hens. The CON hens compared with the PF hens had higher fat pad content at 23 and 28 wk of age, greater ovary and stroma weight at 28 and 32 wk of age, and greater oviduct weight at 28 wk of age. These results showed that the CON hens likely had more resources available for egg production and partitioned more energy toward developing reproductive tissues which advanced the age at 50% production. To provide the practical conclusion whether use the PF system in the industry for the maximum egg production in broiler breeders, the results of the current study should be taken in the context of other research results that used the PF system to feed broiler breeders. The PF system can be used for increasing egg production in broiler breeders. Because it was shown by the previous research in our group that increasing the target BW for 22% compared with the standard breeder-recommended target BW increased egg production. Therefore, we hypothesized that the target BW for the PF birds should be increased before the laying period and around the time of sexual maturation to help the PF birds to increase their ME intake and the body fat deposition to increase their productivity. The ME_e (0.75 kcal/g) predicted from the ME intake model did not have biological meaning which showed that the predicted ME intake model was not realistic. The reasons could be due to the relatively small sample size for the number of experimental units and the short duration of the egg collection.

4.6 Acknowledgments

Financial support from the Alberta Agriculture and Forestry (Edmonton, Alberta, Canada), Alberta Innovates Bio Solutions (Edmonton, Alberta, Canada), Agriculture and Food Council (Edmonton, Alberta, Canada), Alberta Chicken Producers (Edmonton, Alberta, Canada), the Poultry Industry Council (Guelph, Ontario, Canada), Danisco Animal Nutrition (DuPont; Marlborough, Wiltshire, United Kingdom), Canadian Hatching Egg Producers (Ottawa, Ontario, Canada), Alberta Hatching Egg Producers (Edmonton, Alberta, Canada), and the Ontario Broiler Chicken Hatching Egg Producers Association (Guelph, Ontario, Canada) is gratefully acknowledged. In kind support for the precision feeding system was provided by Xanantec Technologies, Inc. (Edmonton, Alberta, Canada). Excellent technical expertise provided by staff and students at the University of Alberta Poultry Research Centre (Edmonton, Alberta, Canada) is gratefully acknowledged.

4.7 References

Abeyrathne, E. D., H. Y. Lee, and D. U. Ahn. 2013. Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. Poult. Sci. 92:3292–3299.

Aviagen. 2011. Ross 308 parent stock: Performance objectives. Aviagen Inc., Huntsville, AL. Accessed Aug. 2018. http://kenanaonline.com/files/0081/81704/Ross-308-PS-PO-2011.pdf.

Aviagen. 2013. Ross 308 parent stock: Nutrition specifications. Aviagen Inc., Huntsville, AL. Accessed Aug. 2018.

http://sedghi.iut.ac.ir/sites/sedghi.iut.ac.ir/files//files_course/ross308ps-nutritionspecs-2013final.pdf.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83:189–204.

Becker, W. A., J. V. Spencer, L. W. Mirosh, and J. A. Verstrate. 1979. Prediction of fat and fat free live weight in broiler chickens using back skin fat, abdominal fat and live body weight. Poult. Sci. 58:835–842.

Bédécarrats**,** G. Y., H. McFarlane, S. R. Maddineni, and R. Ramachandran. 2009. Gonadotropin-inhibitory hormone receptor signaling and its impact on reproduction in chickens. Gen. Comp. Endocrinol. 163:7–11.

Bowmaker, J. E., and R. M. Gous. 1989. Quantification of reproductive changes and nutrient requirements of broiler breeder pullets at sexual maturity. Br. Poult. Sci. 30:663–675.

Canadian Council on Animal Care. 2009. A guide to the care and the use of experimental animals. CCAC, Ottawa, ON, Canada.

Chwalibog, A. 1992. Factorial estimation of energy requirement for egg production. Poult. Sci. 71:509–515.

Claus, R., and U. Weiler. 1994. Endocrine regulation of growth and metabolism in the pig: a review. Livest. Prod. Sci. 37:245–260.

Close, W. H. 1990. The evaluation of feeds through calorimetry studies. Pages 21-39 in Feedstuff evaluation. J. Wiseman and D. J. A. Cole ed. Butterworths, University Press, Cambridge, UK.

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927–1939.

de Beer, M., R. W. Rosebrough, B. A. Russell, S. M. Poch, M. P. Richards, and C. N. Coon. 2007. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. Poult. Sci. 86:1726–1738.

Eitan, Y., E. Lipkin, and M. Soller. 2014. Body composition and reproductive performance at entry into lay of anno 1980 versus anno 2000 broiler breeder females under fast and slow release from feed restriction. Poult. Sci. 93:1227–1235.

Fattori, T. R., H. R. Wilson, R. H. Harms, F. B. Mather, R. D. Miles, and G. D. Butcher. 1993. Response of broiler breeder females to feed restriction below recommended levels. 3. Characterizing the onset of sexual maturity. Poult. Sci. 72:2044–2051.

Gabarrou, G. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. J. Nutr. 127:2371–2376.

Gilbert, A. B. 1971. The ovary. Pages 1163–1208 in The Physiology and Biochemistry of the Domestic Fowl. D. J. Bell and B. M. Freeman, ed. Academic Press, London, UK.

Hadinia, S. H., P. R. O. Carneiro, C. A. Ouellette, and M. J. Zuidhof. 2018. Metabolizable energy partitioning of broiler breeder pullets in skip-a-day and precision feeding systems. Poult. Sci. 97:4279–4289.

Hocking, P. M., A. B. Gilbert, M. Walker, and D. Waddington. 1987. Ovarian follicular structure of white leghorns fed ad libitum and dwarf and normal broiler breeders fed ad libitum or restricted until point of lay. Br. Poult. Sci. 28:493–506.

Hossain, M. A., A. F. Islam, P. A. Iji. 2012. Energy utilization and performance of broiler chickens raised on diets with vegetable proteins on conventional feeds. Asian J. Poult. Sci. 6:117–128.

Johnston, D. W. 1970. Caloric density of avian adipose tissue. Comp. Biochem. Physiol. 34: 827–832.

Joseph, N. S., F. E. Robinson, R. A. Renema, and M. J. Zuidhof. 2002. Responses of two strains of female broiler breeders to a midcycle increase in photoperiod. Poult. Sci. 81:745–754.

Leeson, S., and J. D. Summers. 2000. Broiler breeder production. Pages 137–138 in Nutrition and Feeding. Nottingham University Press, Nottingham, England.

Luiting, P., and E. M. Urff. 1991. Residual feed consumption in laying hens. 1. Quantification of phenotypic variation and repeatabilities. Poult. Sci. 70:1655–1662.

Microsoft, 2010. Accessed Mar. 2019. www.office.microsoft.com.

McLeod, E. S., M. A. Jalal, and M. J. Zuidhof. 2014. Modeling ovarian follicle growth in commercial and heritage Single Comb White Leghorn hens. Poult. Sci. 93:2932–2940.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens. Poult. Sci. 94:1611–1618.

Nangsuay, A., Y. Ruangpanit, R. Meijerhof, and S. Attamangkune. 2011. Yolk absorption and embryo development of small and large eggs originating from young and old breeder hens. Poult. Sci. 90:2648–2655.

Nonis, M. K., and R. M. Gous. 2012. Broiler breeders utilise body lipid as an energy source. S. Afr. J. Anim. Sci. 42:369–378.

NRC. 1981. Nutritional energetics of domestic animals and glossary of energy terms. Second Revised Edition, 1981 ed. National Academy Press, Washington, D.C., USA.

Pishnamazi, A., R. A. Renema, D. C. Paul, I. I. Wenger, and M. J. Zuidhof. 2015. Effects of environmental temperature and dietary energy on energy partitioning coefficients of female broiler breeders. J. Anim. Sci. 93:4734–4741.

Pishnamazi, A., R. A. Renema, M. J. Zuidhof, and F. E. Robinson. 2008. Effect of initial full feeding of broiler breeder pullets on carcass development and body weight variation. J. Appl. Poult. Res. 17:505–514.

Radu-Rusu, R. M., M. G. Usturoi, C. G. Radu-Rusu, and I. VacaruOpris. 2012. Nutritional peculiarities of the hen eggs produced within improved cages or free range alternative systems. Lucrări Ştiinţifice-Seria Zootehnie. 57:201–205.

Reeds, P. J., K. W. J. Wahle, and P. Haggarty. 1982. Energy costs of protein and fatty acid synthesis. Proc. Nutr. Soc. 41:155–159.

Renema, R. A., F. E. Robinson, and M. J. Zuidhof. 2007a. Reproductive efficiency and metabolism of female broiler breeders as affected by genotype, feed allocation, and age at photostimulation. 2. Sexual maturation. Poult. Sci. 86:2267–2277.

Renema, R. A., M. E. Rustad, and F. E. Robinson. 2007b. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. World's Poult. Sci. J. 63:457–472.

Reyes, M. E., C. Salas, and C. N. Coon. 2012. Metabolizable energy requirements for broiler breeder in different environmental temperatures. Int. J. Poult. Sci. 11:453–461.

Rodriguez, A. 2017. Effects of daytime and supplemental light spectrum on broiler breeder growth and sexual maturation. MSc Diss. Univ. Guelph, Guelph, Ontario.

Romero, L. F., M. J. Zuidhof, R. A. Renema, A. Naeima, and F. E. Robinson. 2009a. Characterization of energetic efficiency in adult broiler breeder hens. Poult. Sci. 88:227–235.

Romero, L. F., M. J. Zuidhof, R. A. Renema, F. E. Robinson, and A. Naeima. 2009b. Nonlinear mixed models to study metabolizable energy utilization in broiler breeder hens. Poult. Sci. 88:1310–1320.

Saiful, I. M., M. Fujita, and T. Ito. 2002. Effect of physical activities on heat production of white leghorn hens under different lighting regimes. J. Poult. Sci. 39:159–166.

Sakomura, N. K. 2004. Modeling energy utilization in broiler breeders, laying hens and broilers. Braz. J. Poult. Sci. 6:1–11.

Scott, T. A., and F. Boldaji. 1997. Comparison of inert markers [chromic oxide or insoluble ash (Celite^{TM})] for determining apparent metabolizable energy of wheat or barley–based broiler diets with or without enzymes. Poult. Sci. 76:594–598.

Sharma, V. K., S. S. Kundu, C. Datt, S. Prusty, M. Kumar, U. B. Sontakke. 2018. Buffalo heifers selected for lower residual feed intake have lower feed intake, better dietary nitrogen utilisation and reduced enteric methane production. J. Anim. Physiol. Anim. Nutr. 102:e607–e614.

Swennen, Q., P. J. Verhulst, A. Collin, A. Bordas, K. Verbeke, G. Vansant, E. Decuypere, and J. Buyse. 2007. Further investigations on the role of diet-induced thermogenesis in the regulation of feed intake in chickens: comparison of adult cockerels of lines selected for high or low residual feed intake. Poult. Sci. 86:1960–1971.

Thomas, V. G., S. K. Mainguy, and J. P. Prevett. 1983. Predicting fat-content of geese from abdominal fat weight. J. Wildl. Manage. 47:1115–1119.

van der Klein, S. A. S., G. Y. Bédécarrats, F. E. Robinson, and M. J. Zuidhof. 2018a. Early photostimulation at the recommended body weight reduced broiler breeder performance. Poult. Sci. 97:3736–3745.

van der Klein, S. A. S., G. Y. Bédécarrats, and M. J. Zuidhof. 2018b. The effect of rearing photoperiod on broiler breeder reproductive performance depended on body weight. Poult. Sci. 97:3286–3294.

van Emous, R. 2015. Body composition and reproduction in broiler breeders: impact of feeding strategies. PhD Diss. Univ. Wageningen, Wageningen.

Vogtmann, H., P. Frirter, and A. L. Prabuck. 1975. A new method of determining metabolizability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 16: 531–534.

Yang, S., Y. Suh, Y. M. Choi, S. Shin, J. Y. Han, K. Lee. 2013. Loss of fat with increased adipose triglyceride lipase-mediated lipolysis in adipose tissue during laying stages in quail. Lipids: 48:13–21.

Yang, N., C. Wu, I. McMillan. 1989. New mathematical model of poultry egg production. Poult. Sci. 68:476–481.

Zuidhof, M. J. 2018. Lifetime productivity of conventionally and precision-fed broiler breeders. Poult. Sci. 97:3921–3937.

Zuidhof, M. J. 2019. A review of dietary metabolizable and net energy: Uncoupling heat production and retained energy. Appl. Poult. Res. 28:231–241.

Zuidhof, M. J., M. V. Fedorak, C. C. Kirchen, E. H. M. Lou, C. A. Ouellette, and I. I. Wenger. 2016. System and method for feeding animals. PrecisionZX, Inc., assignee. United States Patent Application No. 15/283,125.

Zuidhof, M. J., M. V. Fedorak, C. A. Ouellette, and I. I. Wenger. 2017. Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity. Poult. Sci. 96:2254–2263.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult. Sci. 93:2970–2982.

4.8 Tables

Table 4-1. Composition and calculated analysis of broiler breeder diet provided from 23 to 34 wk of age.

Ingredient	g/kg
Corn	367
Wheat	350
Soybean meal	158
Canola oil	17
Ground limestone	78
Dicalcium phosphate	14
Choline chloride premix	5
Vitamin premix[1]	2.5
Mineral premix[2]	2.5
NaCl	3.7
D, L-methionine	1.5
L-lysine	0.3
Enzyme[3]	0.5
Total:	1,000
Analyzed composition, as fed basis	
AME (kcal/kg)	2,715
CP (g/kg)	156
Calculated composition, as fed basis	
AME (kcal/kg)	2,909
CP (g/kg)[4]	157
Calcium (g/kg)	33.9
Nonphytate phosphorous (g/kg)	3.7
Available lysine (g/kg)	7.3
Available methionine (g/kg)	4.1
Available methionine + cysteine (g/kg)	6.9

[1]Premix provided per kilogram of diet: vitamin A (retinyl acetate), 12,500 IU; cholecalciferol, 3,125 IU;
Vitamin E (DL- α-tocopheryl acetate), 40.0 IU; vitamin K, 2.50 mg; pantothenic acid, 12.5 mg; riboflavin, 7.50 mg; folacin, 0.63 mg; niacin, 37.50 mg; thiamine, 2.55 mg; pyridoxine, 5.00 mg; vitamin B_{12}, 0.02 mg; biotin, 0.15 mg.
[2]Premix provided per kilogram of diet: iodine, 1.65 mg;
Mn, 88 mg; Cu, 15.0 mg; Zn, 100 mg; Se, 0.30 mg; Fe, 80.0 mg.
[3]Avizyme 1302 feed enzyme for use in poultry diets containing at least 20% wheat (Danisco Animal Nutrition, Marlborough, Wiltshire, UK); minimum activity: 5000 U/g endo-1,4-beta-xylanase, 1600 U/g subtilisin (protease).
[4]Analyzed N using Leco TruMac (Leco Corporation, St. Joseph, Michigan, USA)

Table 4-2. Metabolizable energy intake (MEI), average daily gain (ADG), and body weight (BW) of broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age.

	MEI		ADG		BW			
Period (wk)	PF	CON	PF	CON	Age (wk)	PF	CON	Ross 308 Recommended Target BW
	kcal/hen/d		g/d			g		
	354^{s}	366^{r}	12.6^{s}	16.9^{r}		3,094^{s}	3,284^{r}	-
					23	2,602^{a,Q}	2,477^{b,Q}	2,660
23 to 24	300^{b,MN}	359^{a,KLM}	13.3^{b,KLM}	23.9^{a,J}	24	2,611^{Q}	2,610^{P}	2,820
24 to 25	318^{b,L}	372^{a,IJK}	23.2^{b,IJ}	30.6^{a,IJ}	25	2,718^{P}	2,768^{O}	2,975
25 to 26	357^{b,K}	375^{a,IJ}	28.5^{I}	29.7^{IJ}	26	2,885^{b,O}	2,979^{a,N}	3,120
26 to 27	310^{b,LM}	378^{a,IJ}	16.3^{b,JKL}	31.9^{a,I}	27	3,041^{b,N}	3,194^{a,M}	3,245
27 to 28	289^{b,N}	364^{a,JKL}	19.5^{b,JK}	35.0^{a,I}	28	3,140^{b,M}	3,428^{a,L}	3,340
28 to 29	361^{K}	346^{M}	7.7^{MNO}	9.4^{KL}	29	3,245^{b,L}	3,588^{a,K}	3,395
29 to 30	380^{a,J}	349^{b,M}	3.5^{NO}	7.6^{KLM}	30	3,303^{b,KL}	3,621^{a,JK}	3,435
30 to 31	376^{a,J}	357^{b,LM}	4.0^{NO}	2.5^{LM}	31	3,324^{b,K}	3,637^{a,JK}	3,465
31 to 32	409^{a,I}	365^{b,JKL}	10.2^{LMN}	3.5^{KLM}	32	3,362^{b,JK}	3,669^{a,IJ}	3,490
32 to 33	396^{a,I}	383^{b,I}	11.3^{LM}	10.2^{K}	33	3,428^{b,IJ}	3,709^{a,I}	3,510
33 to 34	399^{a,I}	377^{b,IJ}	1.5^{O}	1.7^{M}	34	3,466^{b,I}	3,727^{a,I}	3,530
Source of variation	SEM	P-value	SEM	P-value		SEM	P-value	
Treatment	2.6	0.006	0.8	0.001		14.9	< 0.001	
Age	4.0	< 0.001	1.8	< 0.001		19.1	< 0.001	
Treatment x Age	5.6	< 0.001	2.6	< 0.001		27.1	< 0.001	

$^{a-b}$ means within row within each variable with no common superscript differ ($P < 0.05$)
$^{I-Q}$ means within column with no common superscript differ ($P < 0.05$)
$^{r-s}$ means within row within overall treatment effect with no common superscript differ ($P < 0.05$)

Table 4-3. Carcass characteristics in broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments at 23, 28, 32, and 34 wk of age.

	Breast				Fat Pad			
	PF	SEM	CON	SEM	PF	SEM	CON	SEM
	% of live BW							
	19.6	0.39	19.6	0.39	1.8^{s}	0.07	2.0^{r}	0.07
Age (wk)								
23	19.7	0.48	19.9^{IJ}	0.41	1.2^{b,J}	0.17	1.7^{a,J}	0.14
28	20.0	0.58	19.1^{J}	0.58	1.9^{b,I}	0.16	2.4^{a,I}	0.16
32	19.1	0.62	19.9^{IJ}	0.62	1.9^{I}	0.16	2.0^{IJ}	0.16
34	19.4	0.33	20.1^{I}	0.43	2.0^{I}	0.06	2.1^{I}	0.11
Source of variation		P-value						
Treatment		0.86				0.003		
Age		0.79				< 0.001		
Treatment x Age		0.046				0.220		

[a-b] means within row within each variable with no common superscript differ ($P < 0.05$)

[I-J] means within column with no common superscript differ ($P < 0.05$)

[r-s] means within row within overall treatment effect with no common superscript differ ($P < 0.05$)

Table 4-4. Hen-day egg production, and egg weight of broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age.

	Hen-day egg production		Egg weight	
	PF	CON	PF	CON
	%		g	
	55.2^{s}	65.5^{r}	57.6	57.4
Period (wk)				
24 to 25	3.8^{P}	6.4^{N}	48.5^{O}	49.6^{O}
25 to 26	9.3^{b,O}	17.4^{a,M}	52.3^{N}	51.8^{N}
26 to 27	27.2^{b,N}	48.7^{a,L}	53.7^{N}	53.9^{M}
27 to 28	52.0^{b,M}	74.1^{a,K}	56.3^{M}	57.6^{L}
28 to 29	69.5^{b,L}	82.4^{a,J}	58.3^{L}	58.5^{KL}
29 to 30	73.4^{b,KL}	85.5^{a,IJ}	59.2^{KL}	58.1^{L}
30 to 31	75.6^{b,JK}	85.0^{a,IJ}	60.4^{JK}	60.8^{IJ}
31 to 32	79.1^{b,IJ}	85.3^{a,IJ}	61.7^{a,IJ}	60.2^{b,JK}
32 to 33	80.6^{b,IJ}	87.6^{a,I}	62.4^{I}	61.3^{IJ}
33 to 34	82.0^{I}	82.9^{IJ}	62.8^{I}	62.0^{I}
Source of variation	SEM	P-value	SEM	P-value
Treatment	1.31	< 0.001	0.25	0.610
Age	1.57	< 0.001	0.47	< 0.001
Treatment x Age	2.22	< 0.001	0.66	0.360

$^{a-b}$ means within row within each variable with no common superscript differ ($P < 0.05$)

$^{I-O}$ means within column with no common superscript differ ($P < 0.05$)

$^{r-s}$ means within row within overall treatment effect with no common superscript differ ($P < 0.05$)

Table 4-5. Carcass characteristics in broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments at 23, 28, 32, and 34 wk of age.

	Ovary and Stroma				Oviduct			
	PF	SEM	CON	SEM	PF	SEM	CON	SEM
	% of live BW							
	1.10^{s}	0.10	1.27^{r}	0.10	1.40^{s}	0.08	1.51^{r}	0.08
Age (wk)								
23	0.02^{L}	0.12	0.02^{K}	0.13	0.01^{K}	0.11	0.01^{K}	0.10
28	$1.02^{b,K}$	0.14	$1.39^{a,J}$	0.14	$1.34^{b,J}$	0.12	$1.72^{a,J}$	0.12
32	$1.41^{b,J}$	0.15	$1.73^{a,I}$	0.15	2.16^{I}	0.12	2.18^{I}	0.12
34	1.96^{I}	0.08	1.95^{I}	0.10	2.08^{I}	0.07	2.13^{I}	0.09
Source of variation	P-value							
Treatment		0.009				0.040		
Age		< 0.001				< 0.001		
Treatment x Age		0.060				0.090		

[a-b] means within row within each variable with no common superscript differ ($P < 0.05$)
[I-K] means within column with no common superscript differ ($P < 0.05$)
[r-s] means within row within overall treatment effect with no common superscript differ ($P < 0.05$)

Table 4-6. Large yellow follicle numbers (LYF), hierarchical follicle weight, hierarchical follicle diameters of breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments at 34 wk of age.

	LYF				
	PF	SEM	CON	SEM	P-value
LYF (n) > 10 mm	6.20	0.12	5.86	0.23	0.18
Hierarchical follicle weight, g					
F1	17.48	0.20	17.34	0.38	0.75
F2	14.68	0.19	14.85	0.36	0.68
F3	11.36	0.22	11.52	0.42	0.74
F4	08.07	0.22	08.09	0.42	0.97
F5	04.93	0.20	05.42	0.38	0.26
F6	02.58	0.14	03.04	0.28	0.14
F7	01.53	0.11	01.60	0.19	0.77

Table 4-7. Total heat production (HP), growth and egg mass (EM), and residual feed intake (RFI) of broiler breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age.

	PF	CON	SEM	P-value
HP (%)[1]	87.7	85.5	0.3	< 0.001
Growth (%)[2]	5.6	7.1	0.3	0.008
EM (%)[3]	6.7	7.4	0.1	0.005
RFI (kcal/d)[4]	6.7	-5.9	2.9	0.009

The following linear model was fitted to derive ME requirements for HP, growth, and hen-day egg production:

$$\text{Observed MEI} = A \times BW^{0.67} + c \times ADG + d \times EM + \varepsilon$$

where MEI was ME intake (kcal/d); A was a vector of age-specific (weekly) total HP coefficients (kcal/kg$^{0.67}$/d); BW was the average BW (kg) of each pen; BW$^{0.67}$ was metabolic BW; c was the coefficient of ADG (g/d); EM was egg mass and d was the coefficient of egg mass (g/d) and the following factors were calculated using the estimates from the above model:

[1] $\text{Total HP\%} = \left[\frac{\text{A} \times \text{Metabolic BW}}{\text{Observed ME intake}}\right] \times 100$

[2] $\text{Growth\%} = \left[\frac{\text{c} \times \text{ADG}}{\text{Observed ME intake}}\right] \times 100$

[3] $\text{Egg mass\%} = \left[\frac{\text{d} \times \text{EM}}{\text{Observed ME intake}}\right] \times 100$

[4] Calculated using the above model:

$$\text{RFI} = \varepsilon = [\text{observed MEI}] - [A \times BW^{0.67} + c \times ADG + d \times EM]$$

Table 4-8. The coefficient of age-specific (A; weekly) total heat production (HP)[1] and the total HP (A × $BW^{0.67}$)[1] of breeder hens on precision feeding (PF) and conventional daily restricted (CON) feeding treatments from 23 to 34 wk of age.

	A		A × $BW^{0.67}$	
	Estimate	SEM	PF	CON
	——— kcal/$kg^{0.67}$/d ———		——— kcal/d ———	
			305^{s}	318^{r}
Period (wk)				
23 to 24	156^{I}	4.3	297^{N}	297^{O}
24 to 25	151^{IJ}	4.9	295^{N}	299^{O}
25 to 26	151^{IJ}	5.0	$307^{b,M}$	$314^{a,N}$
26 to 27	134^{JK}	5.9	$282^{b,O}$	$292^{a,P}$
27 to 28	123^{K}	8.6	$265^{b,P}$	$281^{a,Q}$
28 to 29	134^{IJK}	9.8	$295^{b,N}$	$315^{a,N}$
29 to 30	139^{IJK}	10.2	$310^{b,M}$	$329^{a,M}$
30 to 31	141^{IJK}	10.6	$315^{b,L}$	$335^{a,L}$
31 to 32	145^{IJK}	10.8	$327^{b,J}$	$346^{a,J}$
32 to 33	141^{IJK}	11.0	$322^{b,K}$	$339^{a,K}$
33 to 34	147^{IJK}	11.0	$338^{b,I}$	$355^{a,I}$
Source of variation	P-value		SEM	P-value
Treatment	-		1.0	< 0.001
Age	< 0.001		1.0	< 0.001
Treatment x Age	-		1.5	< 0.001

[1] A was a vector of age-specific (weekly) total HP coefficients and was estimated using a linear model:

$$MEI = A \times BW^{0.67} + 1.75 \times ADG + 0.75 \times EM + \varepsilon$$

Next, A was multiplied by metabolic BW ($BW^{0.67}$) and eventually total HP was estimated using the mixed procedure.

where MEI was observed ME intake (kcal/d); BW was the average BW (kg) of each experimental unit; $BW^{0.67}$ was metabolic BW; c was the coefficient of ADG (g/d), which defined the ME cost for each g of BW gain (kcal/g); EM was egg mass and d was the coefficient of egg mass (g/d), which defined the ME cost for each g of egg mass, and ε was the residual or unexplained error (RFI) and was used to evaluate energetic efficiency.

[a-b] means within row within each variable with no common superscript differ ($P < 0.05$)

[I-Q] means within column with each variable with no common superscript differ ($P < 0.05$)

[r-s] means within row within overall treatment effect with no common superscript differ ($P < 0.05$)

4.9 Figures

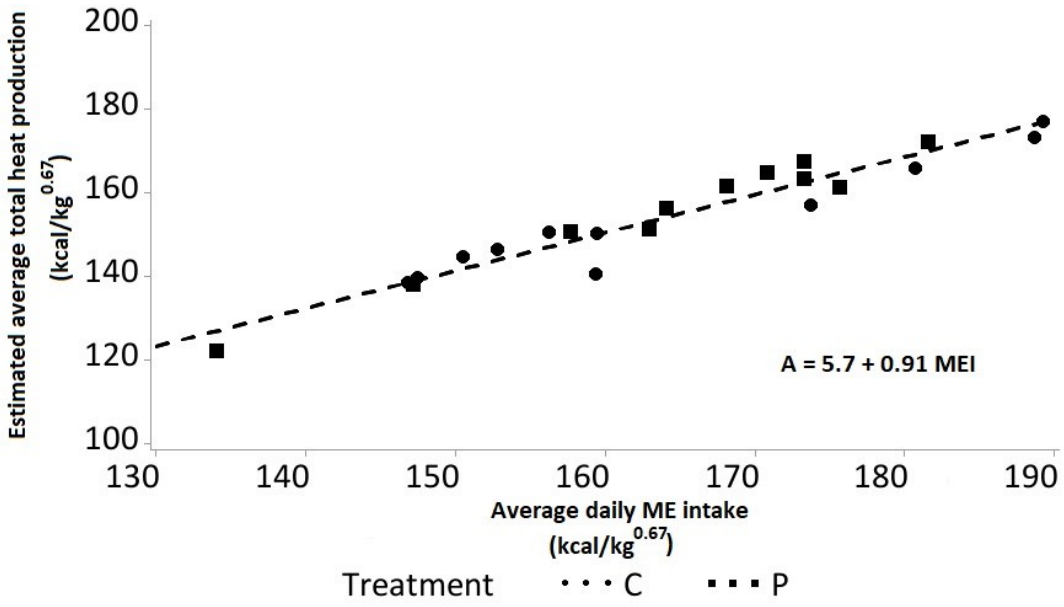

Figure 4-1. Regression of average total heat production [A] versus ME intake ($R^2 = 0.98$; $P < 0.001$). Average daily ME intake (MEI; kcal/kg$^{0.67}$) from 23 to 34 wk of age was calculated for precision feeding (P) and conventional daily restricted feeding (C) treatments. Intercept and slope SEM were 9.30 and 0.06, respectively.

5 Chapter 5: Post-photostimulation Energy Intake Accelerated Puberty in Broiler Breeder Pullets

5.1 Abstract

The effect of ME intake (**MEI**) on the reproductive system was evaluated. Ross 308 broiler breeder pullets (n=140) were assigned to two treatments from 22 to 26 wk of age: 1) Low energy diet fed restricted (2,807 kcal/kg, Low MEI); and 2) High energy diet fed unrestricted (3,109 kcal/kg, High MEI). Daylength was increased from 8 to 14 h at 22 wk of age with a light intensity of 30 lux. Daily palpation was used to detect sexual maturity via the presence of a hard-shelled egg in the shell gland. Expression of gonadotropin releasing hormone-I (**GnRH**) and gonadotropin inhibitory hormone (**GnIH**) genes in the hypothalamus, and GnRH receptor (**GnRH-RI**) and GnIH receptor (**GnIH-R**) genes in the anterior pituitary gland of each pullet was evaluated from 22 to 26 wk of age using quantitative real-time PCR. Blood samples were taken weekly and luteinizing hormone (**LH**), follicle stimulating-hormone (**FSH**) and 17-beta-estradiol (**E2**) determined using commercial ELISA kits. Carcass samples were analyzed for CP and fat content. Data were analyzed using the MIXED procedure in SAS and differences were reported where $P \leq 0.05$. High MEI pullets had 2.3-fold higher GnRH and 1.8-fold higher GnRH-RI mRNA levels than Low MEI pullets. MEI affected neither expression of GnIH and GnIH-R genes nor carcass protein content. For High MEI (489 kcal/d) and Low MEI treatments (258 kcal/d), respectively, from 22 to 26 wk of age ($P < 0.05$), LH concentration was 3.05 and 1.60 ng/mL; FSH concentration was 145 and 89.3 pg/mL; E2 concentration was 429 and 266 pg/mL, and carcass

lipid was 13.9 and 10.3%. The onset of lay for pullets in the High MEI treatment advanced such that 100% had laid by 26 wk of age, compared to 30% in the Low MEI treatment. We concluded that higher MEI advanced the activation of the hypothalamic-pituitary-gonadal axis, increased body lipid deposition, and stimulated reproductive hormone levels which accelerated puberty in broiler breeder pullets.

Key words: caloric restriction, metabolism, gene expression, reproductive hormones, carcass composition

5.2 Introduction

The central nervous system (**CNS**) regulates energy utilization (Richards et al., 2010) and reproduction in avian species (Tsutsui, 2009; Tsutsui et al., 2010b). Some aspects of the energy intake or feed intake regulated by the CNS and peripheral tissues in birds have been assessed previously (Kuenzel, 1994; Kuenzel et al., 1999; Furuse, 2002; Richards, 2003; Furuse et al., 2007; Richards and Proszkowiec-Weglarz, 2007). However, knowledge of the cellular and molecular mechanisms that can be affected by ME intake in poultry is quite limited compared to mammals. The hypothalamus is part of the CNS and plays an important regulatory role for feed intake and energy expenditure by interpreting information from internal physiological signals (hormone and nutrient) and the external environment (photoperiod, temperature, and stressors; Richards and Proszkowiec-Weglarz, 2007). To control energy homeostasis the hypothalamic melanocortin system is composed of two populations of neurons, one set that expresses neuropeptide Y (**NPY**) and a second set that expresses proopiomelanocortin

(**POMC**) that both have important roles in feed intake regulation (Richards et al., 2010). Increased expression of NPY stimulates feed intake and energy storage (anabolic; Kuenzel et al., 1987; Chen et al., 2016), whereas increased expression of POMC reduces energy intake (catabolic; Richards et al., 2010). Moreover, there are other genes that might have an important role in feed regulation. For example, leptin is the product of the obese gene and is released from adipose tissue (Friedman and Halaas, 1998). In mice and humans, injection of leptin results in decreased food intake and decreasing body weight (**BW**; Friedman and Halaas, 1998; Halaas, et al., 1997). Additionally, it was reported that leptin injection advanced the onset of puberty in female rats whose feed intake was less than normal compared to *ad libitum*-fed rats (Cheung et al., 1997). This showed an important link between metabolic status and the reproductive system during puberty in female rats (Cheung et al., 1997). These results indicate that leptin is an important anorexigenic peptide in vertebrates and, along with its receptor, also plays a role in the reproductive system. However, in birds, despite a long standing controversy, the leptin gene was eventually discovered confirming its potential physiological relevance (Seroussi et al., 2016; Friedman-Einat et al., 2014; Prokop et al., 2014; Wang et al., 2016). Moreover, the leptin receptor (**LEPR**) was shown to be expressed within NPY and POMC neurons in the arcuate nucleus of the hypothalamus (Elias et al., 1999).

The hypothalamus also plays a vital role in the reproductive system of birds. The hypothalamus controls the female reproductive cycle and hypothalamic maturation by activating and inhibiting the hypothalamic–pituitary–gonadal (**HPG**) axis (Bédécarrats et al., 2009; Tsutsui et al., 2010a). In the hypothalamus,

stimulatory and inhibitory neuropeptides of the HPG axis are known as gonadotropin releasing hormone (**GnRH**) and gonadotropin inhibitory hormone (**GnIH**) respectively (Bédécarrats et al., 2009). In chickens, two GnRH have been characterized; GnRH-I and GnRH-II (Miyamoto et al., 1982; Miyamoto et al., 1983). Radioimmunoassay measurement of GnRH in the brain of chickens confirmed that GnRH-I is significantly more abundant than GnRH-II in the hypothalamus (Katz et al., 1990). Two specific G-coupled protein receptors have been identified for GnRH-I in anterior pituitary gland (**GnRHR-I** and **GnRHR-II**, Sun et al., 2001; Shimizu and Bédécarrats, 2006). The GnRHR-II is a type III receptor, thus GnRHR-II was renamed to **GnRHR-III** (Joseph et al., 2009). Upon photostimulation, when GnRH binds to its receptors in the anterior pituitary it controls the synthesis and release of luteinizing hormone (**LH**) and follicle stimulating hormone (**FSH**) into the systemic circulation (Robinson and Etches, 1986; Bédécarrats et al., 2009). Both LH and FSH stimulate gametogenesis and the synthesis of sex steroid hormones such as estradiol (**E2**) and they also initiate sexual maturation (Robinson and Etches, 1986; Bédécarrats, 2015; Bédécarrats, 2016). On the other hand, when GnIH is released into the HPG portal vascular system, it binds to its specific G-protein receptor (**GnIH-R**) in the anterior pituitary where it inhibits the production of LH and FSH (Tsutsui et al., 2000; Maddineni et al., 2008; Shimizu and Bédécarrats, 2010). Different possible functions for GnIH have been reported such as regulating reproductive function (Bentley et al., 2008), reproductive behavior (Bentley et al., 2006), and appetite control (Tachibana et al., 2008). As well, it was reported that intra-cerebroventricular (**ICV**) injection of GnIH in

female White-crowned sparrows significantly decreased copulation solicitation (Bentley et al., 2006).

Hypothalamic maturation (age), and achieving BW and carcass compositions thresholds are required for onset of lay in broiler breeders (Renema et al., 1999a). Similarly, laying hens with greater BW and greater lipid content entered into lay earlier than birds with lower BW and lower lipid content (Summers and Leeson, 1983). Thus, it seems that energy balance during sexual maturity can affect the rate of maturation of the neuroendocrine system, which eventually influences the timing of puberty (Renema et al., 1999a).

The first objective of the current study aimed at assessing the effect of ME intake on sexual maturity, specifically on 1) mRNA levels of GnRH-I and GnIH in the hypothalamus and their receptors in the anterior pituitary gland and, 2) the plasma concentration of reproductive hormones (LH, FSH, and E2) in broiler breeders. The second objective was to evaluate the effect of ME intake on the expression of POMC, NPY and LEPR genes to understand how energy balance is regulated by the hypothalamus in broiler breeder pullets at post-photostimulation and during sexual maturity. Finally, the third objective was to investigate the effect of ME intake on carcass composition in broiler breeder pullets. It was hypothesized that higher ME intake by broiler breeder pullets would increase GnRH-I mRNA levels in the hypothalamus and increase the mRNA levels of GnRH receptors in the pituitary to advance the onset of lay. Alternatively, it was hypothesized that higher ME intake by broiler breeder pullets would reduce GnIH mRNA levels in the hypothalamus and suppress the mRNA levels of GnIH-R in the pituitary at the onset

of lay. Moreover, it was hypothesized that the advance in sexual maturation driven by higher mRNA levels of GnRH-I and lower expression of GnIH gene would translate into an increase the concentrations of reproductive hormones after photo-stimulation. Furthermore, it was hypothesized that higher ME intake by broiler breeder pullets would decrease the expression of the NPY gene and increase the expression of POMC and LEPR genes to maintain energy homeostasis. Lastly, it was hypothesized that higher ME intake would increase carcass lipid and decrease carcass protein in broiler breeder pullets at the onset of lay.

5.3 Materials and Methods

5.3.1 Experimental Design

All procedures were approved by the Animal Care and Use Committee for Livestock at the University of Alberta. The focus of the current experiment was sexual maturity and all pullets were fed using a precision feeding (**PF**) system (Zuidhof et al., 2016, 2017). At 21 wk of age, one hundred forty pullets were randomly and equally selected and assigned to two treatments (2 replicate pens with 35 birds each per treatment): 1) Low energy diet fed restricted according to the breeder-recommended Ross 308 BW target (Aviagen, 2011) using a typical commercial diet (2,807 kcal/kg, Low ME intake (Low MEI), Table 5-1) 2) High energy diet fed unrestricted (3,109 kcal/kg, High MEI, Table 5-1). The current experiment was carried out from 22 to 26 wk of age. The target BW for the pullets was interpolated hourly and the High MEI birds were provided access to feed at each visit to the feeding station. At 21 wk, before starting the current experiment, the BW of pullets in the High MEI and in the Low MEI treatments were 2,489 ± 7

and 2,484 ± 7 g, respectively (P > 0.05). Individual birds were considered as the experimental unit since treatments were independently applied to them. The experiment was a completely randomized design and pen was considered as a random effect. Diets were provided in pellet form; the grower diet for the Low MEI treatment was formulated according to breeder recommendations (Table 5-1, Aviagen, 2013) and the grower diet for the High MEI treatment was as the same as the Low MEI diet and contained the same ingredients as the Low MEI treatment except that the amount of canola oil was higher to increase the energy level.

5.3.2 Diet Analyses

The AME content of the diets was determined by adding 2% acid-insoluble ash marker (Celite, Celite 281, Lompoc, CA). Four birds in each pen (8 per treatment) were randomly selected at each of 22, 23, 24, 25 and 26 wk of age and euthanized by cervical dislocation. Ileal digesta samples were collected by gently squeezing the excised intestinal tract from Meckel's diverticulum to the ileal-cecal-colon junction. Digesta samples were pooled for each experimental pen, and stored at -20°C until analysis. Diet and ileal digesta samples were oven dried at 60°C for 48 h and then ground. For acid insoluble ash, samples were digested with 4N HCL and then the residues were ashed at 500 °C (Vogtmann et al., 1975). Using bomb calorimetry, gross energy (**GE**) of feed and digesta samples were measured. The AME values were calculated as previously described by Scott and Boldaji (1997):

$$\mathrm{AME} = \mathrm{GE}_{\mathrm{diet}} - \mathrm{GE}_{\mathrm{digesta}} \times \frac{\mathrm{Marker}_{\mathrm{diet}}}{\mathrm{Marker}_{\mathrm{digesta}}}$$

where GE = gross energy (kcal/kg of sample); and Marker = concentration of acid insoluble ash in sample. Apparent ME values were expressed on an as-fed basis. Nitrogen content of feed was determined by the combustion method using a Leco TruMac N machine (Leco Corporation, St. Joseph, Michigan, USA) and dietary CP was estimated using a factor of 6.25 (Hossain et al., 2012; Mutucumarana et al., 2015).

5.3.3 Management

Each pen contained pine shavings as litter at a depth of approximately 5 cm. The stocking density was 2.1 birds/m^2. Two suspended nipple drinkers (2.5 pullets per nipple) provided water *ad libitum* throughout the experiment. The feeder in each PF station was 4.8 cm wide, and only one bird was provided access at a time. Temperature, measured hourly, was 21 ± 0.40°C during the entire experimental period. Daylength was increased from 8 to 14 h at 22 wk of age with a light intensity of 30 lux. The design and function of the PF stations are fully disclosed elsewhere (Zuidhof et al., 2016; Zuidhof et al., 2017). Prior to start the current experiment birds were trained to become familiarized with the PF stations as explained by Zuidhof (2018). All birds were identified with a unique radio frequency identification (**RFID**) wing tag to be tracked by the PF system and were fed individually based on their BW. Briefly, each pullet was weighed by a built-in platform scale when it entered the PF station. If its BW was equal to or greater than the target BW, the pullet was gently ejected by the station. However, if its BW was lower than the target BW, the PF station provided access to approximately 25 g of feed for one minute, after which the pullet was ejected from the station. Feed intake

was calculated as the initial minus the final feed weight. For each visit, RFID, BW, and initial and final feed weight data were written to a database with a date and time stamp. Target BW was interpolated hourly. The target BW for the Low MEI treatment was based on the breeder-recommended BW target and for the High MEI treatment was set at 10 kg to allow the High MEI pullets to get access to feed whenever they entered to the stations. Thus, pullets could access feed 24 hours per day. Number of meals per day for the High MEI pullets and the Low MEI pullets were 24.81 ± 0.77 and 9.89 ± 0.53 respectively ($P < 0.001$). The meal size for the High MEI pullets and the Low MEI pullets were 6.97 ± 0.18 and 11.76 ± 0.16 g/meal respectively ($P < 0.001$).

5.3.4 Sample Collection

5.3.4.1 Dissection

From 22 to 26 wk of age, 8 birds per treatment per wk were euthanized and dissected. In addition to the 8 birds per treatment, any pullet that entered into lay (had an egg in the shell gland) were euthanized and dissected for the sample collection. Palpation was performed every morning to detect sexual maturity via the presence of a hard-shelled egg in the shell gland.

5.3.4.2 Hypothalamus and Pituitary Tissue Collection

At each of 22 and 23 wk of age, 4 birds per treatment were randomly selected to collect hypothalamus and pituitary tissue samples. At 24, 25, and 26 wk of age, 4 birds per treatment that had, and 4 birds per treatment that had not laid an egg were randomly selected for tissue collection. The hypothalamus and the pituitary samples were immediately collected after cervical dislocation and

dissection, and immediately snap frozen in liquid nitrogen then stored at -80°C until RNA extraction. These samples were used for gene expression analyses.

5.3.4.3 Blood Collection

Blood samples were collected weekly from all birds and approximately 2 mL of blood was taken by venipuncture from the brachial vein and collected in a sodium heparin blood vacutainer tube (Evacuated glass tubes, Fisher Scientific, NH, USA). Blood plasma was recovered by centrifugation at 1244 × g for 15 min at 4°C and stored at −20°C until hormone assay. The same subsample of birds used for gene expression analyses was chosen for hormone assay.

5.3.4.4 Carcass Collection

Each individual bird carcass was pressure-cooked for 2 h and homogenized using an industrial blender. The same subsample of birds used for gene expression analyses and hormone assays was chosen for carcass composition analyses.

5.3.5 Lab Analyses

5.3.5.1 RNA Isolation, cDNA Synthesis, and Real-time Quantitative PCR

Two hundred fifty to 350 mg of hypothalamus sample was homogenized using trizol and total RNA was extracted using Direct-zol™ RNA miniPrep plus kit (Zymo Research, Inc., Irvine, CA, USA). Total RNA of hypothalamus sample was eluted in 40 µl nuclease-free water (Ambion, Austin, TX, USA). Total RNA was extracted from 10 to 15 mg of pituitary sample, using Absolutely RNA miniprep kit (Agilent Technologies, Inc., Palo Alto, CA, USA). Total RNA of pituitary sample was eluted in 24 µl elution buffer provided by the kit. The RNA extraction procedure for both kits was performed according to the manufacturer's

instructions and included a DNase treatment on the purification column. Quantity and purity of total RNA isolated from all samples were determined using a NanoDrop™ 2000c Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Ratio of absorbance at 260 nm and 280 nm of RNA for all samples were between 1.8 and 2.0. Total RNA integrity (RIN) was evaluated using an Agilent 2200 TapeStation (Agilent, Santa Carla, Ca, USA). To evaluate the RIN values for hypothalamus and pituitary samples, RNA of pullets within each treatment was selected randomly from 22 to 26 wk of age. The RIN value of RNA was above 8.5 and 6.5 for hypothalamus and pituitary samples, respectively. Total RNA (100 ng) from each individual hypothalamus and pituitary sample was reverse transcribed to cDNA with SuperScript® VILO™ Master Mix (Invitrogen, Carlsbad, CA, USA) in a final volume of 20 µl. The mixture was incubated for 10 min at 25°C, 60 min at 42°C, 5 min at 85°C, and cooled at 4°C. After reverse transcription, the cDNA was diluted 3 times with nuclease-free-water (1:3, Ambion, Austin, TX, USA). Real-time PCR analysis was performed in duplicate. Two µl of the cDNA was used as the template in a 20 µl of PCR reaction of Power SYBR Green Master Mix (Applied Biosystems., Inc). Thermal PCR cycler was the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, USA), and the amplification program was 95°C for 20 seconds, 95°C for 3 seconds, and 60°C for 30 seconds then repeated 40 cycles. The PCR primers were designed for each gene transcript investigated using Integrated DNA Technologies PrimerQuest® tool (http://www.idtdna.com/Primerquest/Home/Index). Specificity of the primer sequences (*Gallus gallus*) was then confirmed using Basic Local Alignment

Search Tool (BLAST) at National Centre for Biotechnology Information (NCBI; Table 5-2). The amplification efficiencies of all genes were determined using serial dilution of hypothalamus and pituitary samples cDNA (1, 1:5, 1:10, 1:50, 1:100, 1:500, and 1:1000). The amplification efficiencies for all genes in both tissue types were above 95% and slopes of the plots from the serial dilutions were close to -3.32.

Real-time PCR efficiencies were calculated from the given slopes in Step-one plus software. The corresponding real-time PCR efficiency (**E**) of one cycle in the exponential phase was calculated according to the equation: $E = 10^{[-1/slope]}$ (Pfaffl, 2001). Relative expression ratio of a target gene was calculated based on the E and threshold cycle (**Ct**) deviation of control sample and expressed relative to the geometric mean of two stable housekeeping genes (Pfaffl, 2001). The minimum Ct value for each gene was used as the control sample in the current study. Five housekeeping genes; glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**); Actin beta (**ACTB**); succinate dehydrogenase complex flavoprotein subunit A (**SDHA**); ribosomal protein L19 (**RPL19**); eukaryotic translation elongation factor 1 alpha 1 (**EEF1A1**); were tested for the hypothalamus and pituitary samples to select the most stable housekeeping genes in each tissue, and normalize target gene expression from hypothalamus and pituitary samples respectively. Two stable housekeeping genes; ACTB and SDHA were selected to normalize expression measured in hypothalamus samples, and housekeeping genes ACTB and EEF1A1 were selected to normalize expression measured in pituitary samples, using the NormFinder Excel Add-In (Andersen et al., 2004).

5.3.5.2 Hormone Assay

Plasma LH concentration was determined using a commercially available ELISA kit (E-EL-Ch1569, chicken LH, Elabscience Biotechnology, Hubei, China) according to the manufacturer's instructions. The assay was performed in duplicate for each individual sample. The optical density was measured with a microplate spectrophotometer at 450 nm (Molecular Devices, California, USA). The standard curve and samples were plotted and analyzed using SoftMax Pro software. The intra- and inter-assay CV for LH were respectively 8.15 and 6.96%. Plasma FSH concentration was quantified using FSH ELISA kit (E-EL-Ch1365, chicken FSH, Elabscience Biotechnology, Hubei, China) according to the manufacturer's instructions. Assay was performed in duplicates for every individual sample. The intra and inter-assay coefficients of variation for FSH were respectively 8.66 and 10.42%.

To quantify plasma E2 concentration, prior to ELISA assay, E2 was extracted from plasma using ethanol and according to the method suggested by Baxter et al. (2014). Briefly, thawed samples were diluted with ethanol at 5:1 (ethanol: plasma) ratio. Samples were then vortexed, centrifuged for 5 min at 20°C at 1,800 × g, and frozen at -80°C. The organic (ethanol) phase was recovered and transferred into new tubes, and dried using a SpeedVac (Thermo Savant SpeedVac SC210A Centrifugal Evaporator, Thermo Scientific, USA). Samples were reconstituted in half the original volume with assay buffer and stored at -20ºC until assay. Plasma E2 was quantified using E_2 ELISA kit (E-EL-0065, Elabscience Biotechnology, Hubei, China) according to the manufacturer’s instructions. The

assay was performed in duplicate for each individual sample. The optical density was measured with a microplate spectrophotometer at 450 nm (Molecular Devices, California, USA). The standard curve and samples were plotted and analyzed using SoftMax Pro software. The intra and inter-assay coefficients of variation for E2 were respectively 8.51 and 12.79 %.

5.3.5.3 Carcass Composition

200-mL samples of the homogenate were collected and oven-dried at 55°C for 96 h and reground for homogeneity. A digital scale was used to calculate the difference (in milligram (0.001 g)) between the weight of the sample after oven drying and the weight of the sample before oven drying was multiplied to 100 to calculate the dry matter percentage of the sample. Carcass fat was extracted using AOAC 960.39 method (Leffler et al., 2008) and determined by Goldfisch method; diethyl ether ethyl was used as a solvent. Approximately 4 g samples were added to pre-weighed extraction thimbles. Samples were dried in a 100°C drying oven for 24 h and after they cooled, dry weight was recorded. Samples were then placed into a Labconco Goldfish ether extraction apparatus (Serial # 3500100, Kansas City, MO). Approximately 50 mL of diethyl ether per sample was used for fat extraction. Ether was allowed to drip through samples for 6 h. Samples were dried at 100°C in drying oven for 90 min and cooled to room temperature at which point weights were recorded. Fat percentage was determined indirectly by weight loss. Carcass nitrogen was determined by the combustion method using a Leco TruMac N determinator (Leco Corporation, St. Joseph, Michigan, USA) and carcass CP was estimated using a factor of 6.25 (Hossain et al., 2012; Mutucumarana et al., 2015).

5.3.6 Statistical Analysis

5.3.6.1 Pearson Correlation

The Pearson correlation coefficient was estimated to measure the strength of the linear relationship between the expression of genes. The Pearson correlation was estimated in overall considering all birds and also it was estimated within individuals that had laid or had not laid an egg. The Pearson correlation was estimated using the CORR procedure in SAS software (version 9.4; SAS Inst. Inc., Cary, NC). 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 $P \leq 0.05$.

5.3.6.2 Analysis of Variance

Treatment effects for the mRNA levels of all genes (GnRH-I, GnIH, GnRH-RI, GnRH-RIII, GnIH-R, LH, FSH, POMC, NPY, and LEPR), the ratio of GnRH-RI to GnRH-RIII; the ratio of GnRH-RI to GnIH-R; the ratio of GnRH-RIII to GnIH-R; BW; ME intake; reproductive hormones (LH, FSH, E2); carcass compositions; and percentage of flock in lay were evaluated using 2-way ANOVA using the MIXED procedure in SAS, with age and treatment as fixed effects. Moreover, since birds started to lay eggs at different ages from 24 wk onward, the presence of an egg was included as a covariate for the mRNA levels of all genes; the ratios of GnRH-RI to GnRH-RIII, GnRH-RI to GnIH-R, and GnRH-RIII to GnIH-R; reproductive hormones; and carcass composition. The covariate took the value of either 0 or 1, respectively, for birds that did not or did enter into lay, and

estimated the magnitude of the change in the expected value for each variable with respect to reproductive status. The PDIFF option of the LSMEANS statement used to estimate pairwise differences between means and least significant difference test was applied to multiple mean comparisons. Differences between means were reported as significant where $P \leq 0.05$. Trends were reported where $P < 0.1$.

5.4 Results and Discussion

5.4.1 Metabolizable Energy Intake, and BW

Since the High MEI treatment birds were fed whenever they entered the stations, it was not surprising that the High MEI pullets had higher ME intake compared to the Low MEI pullets during the entire experimental period (Table 5-3). Higher ME intake of pullets in the High MEI treatment resulted in greater BW relative to the pullets in the Low MEI treatment from 22 to 26 wk of age (Table 5-3). Pearson and Herron (1981) explained that surplus energy intake is mainly stored as fat. In the current experiment, the High MEI pullets with higher ME intake than the Low MEI pullets partitioned extra nutrients for carcass lipid deposition (it will be discussed further in the next sections) and also for the ovary development. The High MEI pullets compared to the Low MEI pullets had greater ovary weight as a percentage of BW from 22 to 26 wk of age (0.62% ± 0.07 and 0.32% ± 0.07 respectively; $P = 0.003$; data not shown). The greater ovary weight of the High MEI pullets indicates that they partitioned more energy toward developing reproductive tissues for the onset of egg production than the Low MEI pullets, and this is consistent with observed egg production data (Table 5-3).

5.4.2 Gene Expression

5.4.2.1 Effect of ME Intake on Genes of the Reproductive Axis

The High MEI treatment had a higher mRNA level of GnRH-I gene compared with the Low MEI treatment at 23 wk of age (1.77 vs. 0.63 respectively, Table 5-4), and overall from 22 to 26 wk of age (0.74 vs. 0.32 respectively, Table 5-4). Moreover, the High MEI treatment had also a higher mRNA level of GnRH-RI gene compared with the Low MEI treatment at 25 and 26 wk of age and overall from 22 to 26 wk of age (2.84 vs. 1.54 respectively, Table 5-4). The mRNA level of GnRH-I gene increased at 23 wk of age compared to other ages, however, the mRNA level of GnIH decreased with age from 23 to 26 wk of age (Table 5-4). The mRNA level of GnRH-RI gene reached the maximum at 25 and 26 wk of age (Table 5-4). Similarly, the mRNA level of LH (beta-subunit) gene increased with age from 22 to 26 wk of age. Interestingly, mRNA level of FSH (beta-subunit) gene was greater at 22 and 23 wk of age compared to other ages (Table 5-5). On the other hand, the High MEI and the Low MEI treatments did not impact mRNA levels of GnIH, GnRH-RIII, GnIH-R, LH, and FSH genes from 22 to 26 wk of age (Table 5-4). The ratio of GnRH-RI to GnRH-RIII was 1.63 times higher in the High MEI than the Low MEI treatment from 22 to 26 wk of age (Table 5-6).

Moreover, the ratio of GnRH-RI to GnIH-R mRNA was 1.38 times higher in the High MEI than the Low MEI treatment from 22 to 26 wk of age (Table 5-6). However, the ratio of GnRH-RIII to GnIH-R mRNA was not significantly different between the High MEI and the Low MEI treatments but a trend showed that this ratio was 1.41 times higher in the Low MEI than the High MEI treatment from 22

to 26 wk of age (Table 5-6). For the mRNA level of every gene, the covariate egg effect was estimated (Table 5-4 and Table 5-5). Without considering the egg effect as the covariate, the relative expression of the genes would be biased systematically. If an egg had been laid the relative expression of GnRH-I, GnIH, GnRH-RIII, and LH were different by 0.34, 0.48, 0.38, and -1.03 respectively.

In birds, reproduction is mainly regulated by stimulatory GnRH-I and inhibitory GnIH hypothalamic neuropeptides, upon interaction with their receptors in the anterior pituitary (Bédécarrats et al. (2009). Photostimulation increases the mRNA level of GnRH-I in the hypothalamus of chickens (Bédécarrats et al., 2006). However under short day length; the mRNA level of GnIH gene is increased in quails (Ubuka et al., 2005). In the current experiment, in addition to higher mRNA level of GnRH-I for the High MEI treatment compared to the Low MEI treatment, the mRNA level of GnRH-RI was also higher in the High MEI treatment from 22 to 26 wk of age. Moreover, the ratio of GnRH-RI to GnRH-RIII was 1.6 times higher in the High MEI treatment than the Low MEI treatment. This indicated that GnRH-I interacted with GnRH-RI and caused the higher mRNA level of GnRH-RI in the High MEI treatment compared to the Low MEI treatment. Interestingly, it was previously shown that GnRH-RI is barely expressed in the pituitary and GnRH-RIII expression in pituitary was 1373-fold that of GnRH-RI in broiler chickens (Joseph et al., 2009). Similarly, a significant increase in White Leghorn birds GnRH-RIII mRNA in pituitary only after photostimulation was reported, whereas levels of GnRH-RI mRNA were found to be constant regardless of reproductive stage (Shimizu and Bédécarrats, 2006). In the current study, it seems that high ME

intake along with photostimulation increased GnRH-RI expression while Joseph et al. (2009) and Shimizu and Bédécarrats (2006) found that photostimulation increased GnRH-RIII expression. In the current study, the greater ratio of GnRH-RI to GnIH-R and the lower ratio of GnRH-RIII to GnIH-R in the High MEI treatment than the Low MEI treatment showed that the ME intake changed the ratio of GnRH-RI to GnIH-R in favor of GnRH-RI from 22 to 26 wk of age by sexual maturity and the onset of lay.

There was a sharp increase in the mRNA level of GnRH-I gene at wk 23 compared to other ages, which is most likely due to photostimulation. Photostimulation was started at the beginning of wk 22 and it is consistent with the hypothesis that broiler breeders are the most responsive (or estrogenic) to follicle development between 2 to 4 wk after photostimulation (Robinson et al., 2003). Increased LH expression with age and the greater FSH expression at 22 and 23 wk of age were also consistent with photostimulation and maximum responsiveness of GnRH-I gene between 2 to 4 wk after photostimulation. We hypothesized that GnRH-RI may be more prevalent on the gonadotropes synthesizing LH as the increased expression of LH and GnRH-RI genes occurred concomitantly with age. Therefore, these observations indicated that the expression of GnIH and GnRH-I in the hypothalamus might shift toward increasing GnRH-I with age and by sexual maturity and the onset of lay.

5.4.2.2 Effect of ME intake on Genes Involved in the Regulation of Energy Balance

The mRNA levels of NYP and LEPR did not differ between the High MEI and the Low MEI treatments from 22 to 26 wk of age (Table 5-5). On the other hand, the mRNA level for POMC was greater in the High MEI treatment compared to the Low MEI at 22 and 23 wk of age (Table 5-5).

AMP-activated protein kinase (**AMPK**) is a serine-threonine kinase and regulates cellular metabolism (Carling, 2004; Proszkowiec-Weglarz et al., 2006a; Proszkowiec-Weglarz and Richards, 2007). AMPK maintains energy homeostasis and regulates food intake by changing the expression of orexigenic (stimulates feeding behavior) neuropeptides such as NPY and anorexigenic (inhibits feeding behavior) neuropeptides such as POMC in the hypothalamus (Long and Zierath, 2006). Activation of AMPK in the hypothalamus stimulates energy producing pathways (catabolic) results in oxidation of glucose and fatty acids and it inhibits energy-consuming pathways (anabolic; Proszkowiec-Weglarz et al., 2006a,b; Proszkowiec-Weglarz and Richards, 2007; Winder and Thompson, 2007). Moreover, activation of AMPK in the hypothalamus stimulated the activity of the NPY expressing neuron (anabolic) and resulted in increasing feed intake and reduced energy expenditure which consequently increased energy status in response to lowered energy status (Long and Zierath, 2006; Minokoshi et al., 2004). However, in the current experiment, NPY expression did not differ between treatments. Moreover, in the current experiment, the mechanism for down-regulation of POMC expression by the Low MEI was not assessed. It appears that

down-regulation of POMC expression in the Low MEI treatment compared to the High MEI treatment occurred via AMPK activation. The AMPK activation in the Low MEI treatment was probably due to enhance catabolic pathways and inhibit anabolic pathways.

The LEPR in birds has been found in the hypothalamus (Horev et al., 2000; Paczoska-Eliasiewicz et al., 2003), in the pituitary (Paczoska-Eliasiewicz et al., 2003) and in the ovary (Ohkubo et al., 2000; Paczoska-Eliasiewicz et al., 2003). This may suggest a role for leptin in reproductive system activity. In humans, ICV injection of leptin decreased food intake, decreased adipose tissue and decreased BW (Friedman and Halaas, 1998). Similarly, ICV injection of leptin suppressed feed intake in broiler chickens and in Leghorns (Denbow et al., 2000). The results of the current experiment showed that although LEPR was expressed in the hypothalamus of broiler breeder pullets from 22 to 26 wk of age, ME intake was not different between the Low MEI and the High MEI treatments.

5.4.3 The Correlation between the Genes

Overall, GnRH-I and GnIH mRNA levels were positively correlated ($r = 0.36$; $P = 0.01$). This positive correlation between GnRH-I and GnIH gene expression was observed within individuals that entered lay ($r = 0.42$; $P = 0.05$) as well as within individuals that had not ($r = 0.37$; $P = 0.03$). Similarly, the overall expression of the POMC and GnRH-I genes were positively correlated ($r = 0.31$; $P = 0.04$) both within individuals that entered lay ($r = 0.38$; $P = 0.08$) and within individuals that had not laid ($r = 0.31$; $P = 0.08$). Moreover, expression of the POMC and GnIH genes were also positively correlated overall ($r = 0.50$; $P = 0.005$)

within individuals that had laid ($r = 0.86$; $P < 0.001$) or not ($r = 0.49$; $P = 0.004$). Interestingly, overall the mRNA levels of POMC were inversely related to the mRNA levels of LH ($r = -0.40$; $P = 0.006$), within individuals that entered lay ($r = -0.61$; $P = 0.002$) and within individuals that did not ($r = -0.48$; $P = 0.005$).

Positive correlation of GnIH and GnRH-I gene expressions suggests some relationship is maintained between GnRH-I and GnIH neurons. Within the hypothalamus, GnIH neurons directly contact GnRH-I neurons suggesting that GnIH may directly regulate the synthesis and release of GnRH-I at the level of the cell body (Bentley et al., 2003). Positive correlation of POMC with GnRH-I and GnIH, and the negative correlation of POMC with LH provides a new insight for POMC function. It is hypothesized that POMC may link the control of reproduction with the control of energy status in broiler breeders. The POMC gene possibly signals to modulate GnRH, GnIH, and LH gene expressions and integrate this information with energy storage information to allow or prevent ovulation to proceed.

5.4.4 Reproductive Hormones

The concentration of plasma LH was higher in the High MEI treatment compared to the Low MEI treatment at 22 wk of age (7.10 vs. 0.93 ng/mL; Table 5-7). Similarly, the concentration of plasma FSH was higher in the High MEI treatment compared to the Low MEI treatment at 22 wk of age (336 vs. 90.6 pg/mL, Table 5-7). The overall concentration of plasma E2 was higher in the High MEI treatment relative to the Low MEI treatment from 22 to 26 wk of age (429 vs. 266 pg/mL, Table 5-7). The LH and FSH plasma concentrations decreased with age and

their levels decreased sharply after wk 22 (Table 5-7). The E2 plasma concentration was highest at 25 wk of age compared to the other ages for both ME intake treatments (Table 5-7) indicating that timing of the activation of the ovarian follicular pool was not affected but rather the amplitude was increased under high ME intake. For the concentration of each reproductive hormone, the covariate egg was estimated and it was not significant (Table 5-7). This indicated that reproductive status did not affect the concentrations of the reproductive hormones from 22 to 26 wk of age.

The major source of E2 is small white follicles in the ovary of a hen however large white follicles and small yellow follicles also produce E2 in small amounts (Robinson and Etches, 1986). Moreover, E2 is produced from theca cells of large yellow follicles in small amounts (Etches and Duke, 1984; Robinson and Etches, 1986). The greater concentration of plasma E2 for the High MEI birds relative to the Low MEI birds suggests more pronounced ovary development in the High MEI birds. It was consistent with the results of the ovary weight as a percentage of live BW that the High MEI treatment had higher ovary weight than the Low MEI treatment from 22 to 26 wk of age (0.62% ± 0.07 and 0.32% ± 0.07 respectively; P = 0.003; data not shown). Estradiol increased with age in *ad libitum* fed broiler breeder hens and peaked at 25 wk (Onagbesan et al., 2006). In restricted broiler breeder hens, the concentration of E2 at peak was significantly lower than the *ad libitum* fed birds (Onagbesan et al., 2006). Similarly, in the current experiment, the E2 peaked at 25 wk of age (age effect) and pullets in the Low MEI treatment had lower plasma E2 levels compared to the High MEI pullets. In the current

experiment, the peak of E2 concentration occurred after the peaks of the gonadotropins and this indicated that the gonadotropins drove the E2 secretion. Similarly, it was observed in previous studies in broiler breeder hens that peak of E2 concentration happened after peaks of the gonadotropins (Onagbesan et al., 2006, Renema et al., 1999b). A similar result was reported in Leghorn hens (Imai and Nalbandov, 1978).

In the current experiment, the overall higher plasma LH and FSH concentrations in the High MEI treatment relative to the Low MEI treatment showed that the ME intake enhanced reproductive development as the ovary weight was higher in the High MEI treatment than the Low MEI treatment. Interestingly, considering the age effect, the peaks of FSH coincided with the peaks of LH at 22 wk of age. This suggested that there was a synergistic effect between LH and FSH as reported by Imai and Nalbandov (1978) and that both could be stimulated by GnRH. Plasma LH and FSH concentrations increased to peak levels within 3 d of photostimulation and subsequently declined as sexual maturity proceeded in broiler breeder hens (Renema et al., 1999b). It was reported that FSH peak occurred at 20 wk in *ad libitum* fed birds when photostimulation was started at 19 wk of age (Liu et al., 2015). In the current experiment, photostimulation was started at the beginning of wk 22 and the plasma LH and FSH peaked at the end of wk 22 (6 d post-photostimulation) and subsequently, their values declined with age as the plasma E2 increased with age. Similarly, plasma LH concentration declined with age in White Leghorn chickens as the plasma steroid concentration increased with age (Vanmontfort et al., 1995). Renema et al. (1999b) suggested that the reduction

in plasma LH and FSH after the peak could be due to the negative feedback effects of E2 on hypothalamic stimulation of LH and FSH. It was shown that in humans, E2 negative feedback on plasma LH and FSH occurs at the hypothalamus level (Welt et al., 2003) and at the pituitary level (Shaw et al., 2010). However, in broiler breeders there is lack of information whether E2 has a direct negative feedback effect on plasma LH and FSH either at the hypothalamus or at the pituitary level. Sun et al. (2001) suggested that estrogen played an inhibitory effect on hypothalamic neurons by reducing GnRH-I mRNA in cockerels. Maddineni et al. (2008) reported that estrogen decreased pituitary GnIH-R mRNA levels in female White Leghorns and GnIH-R gene probably mediated inhibitory effect of GnIH on LH and FSH secretion. It was shown in mice that E2 has a direct inhibitory effect on plasma LH at the hypothalamus level (Couse and Korach, 1999). Moreover, it was reported that in the ewe (Clarke and Cummins, 1984), and rat E2 has a direct inhibitory effect on plasma LH and FSH at the pituitary level (McLean et al., 1975). In the current study, plasma E2 probably had a negative feedback effect on the stimulation of LH and FSH either at the hypothalamus or pituitary level.

5.4.5 Carcass Composition

The High MEI and the Low MEI birds did not differ in protein, ash, and water content from 22 to 26 wk of age. However, pullets in the High MEI treatment had higher lipid than the pullets in the Low MEI treatment from 23 to 26 wk of age (Table 5-8). Overall, protein increased with age and fat also increased with age whereas water decreased with age (Table 5-8). A non-significant covariate

indicated that reproductive status did not affect the estimation for the carcass compositions from 22 to 26 wk of age.

Pullets in the High MEI treatment had higher ME intake and greater BW compared to the pullets in the Low MEI treatment. Extra energy intake is mainly stored as fat (Pearson and Herron, 1981), thus higher lipid content of pullets in the High MEI treatment may be related to the difference in ME intake and BW. Lipid contains more energy (9.1 kcal/g; Johnston, 1970) compared to other energy sources. Pullets in the High MEI treatment had advanced onset of lay relative to the pullets in the Low MEI treatment because they had more energy available in the body and sufficient metabolic status to stimulate the onset of lay. It was reported that BW and lipid percent of BW for *ad libitum*-fed broiler breeder birds were greater than feed-restricted pullets at sexual maturity and sexual maturity was advanced in the *ad libitum*-fed birds than the feed-restricted birds for 13.6 d ($P < 0.05$, Renema et al., 1999a).

In the current experiment, pullets had higher lipid and protein at wk 26 compared to wk 22. This indicated that broiler breeder pullets partitioned more energy toward protein and lipid deposition with age as sexual maturation proceeded. Lipid and protein contain more energy compared to the water (9.1, 5.5, and 0 kcal/g respectively; Johnston, 1970; Pullar and Webster, 1977) and the pullets required to partitioned energy toward egg production as sexual maturity proceeded. Therefore, the metabolism of pullets changed with age, toward increasing protein and fat deposition.

5.4.6 The Onset of Sexual Maturity

All pullets in the High MEI treatment entered lay by 26 wk of age however only 30% of the pullets in the Low MEI treatment entered lay by 26 wk of age (Table 5-3). Heavier laying hens with greater lipid content have been shown to enter lay earlier (Summers and Leeson, 1983). It was also reported that broiler breeder hens from 1980 with 5.38% abdominal fat pad entered lay 19.2 d earlier than hens from 2000 and with 2.65% of abdominal fat pad (Eitan et al., 2014). Similarly, in the current experiment, in the High MEI treatment relative to the Low MEI treatment, lipid carcass was 1.3 times higher from 22 to 26 wk of age, thus the High MEI pullets had more energy available to start the onset of sexual maturity. It was recently shown that modern broiler breeder pullets restricted fed according to the standard breeder-recommended target BW mostly deposited lean tissues (Hadinia et al., 2018). Interestingly, although the growth potential of broiler breeders increased during the last 30 years (Renema et al., 2007), feed restriction became more severe with continued selection program for broiler growth traits (van Emous, 2015). Severe feed restriction decreases the nutrients available in the body such as fat, which eventually affects the body composition and delays the onset of lay in modern broiler breeders. It was reported that broiler breeder hens that followed the standard breeder-recommended target BW delayed the onset of lay for 18.9 d compared to hens with higher target BW (target BW increased by 22% reaching the standard 21 wk BW at 18 wk; van der Klein et al., 2018). Moreover, total egg production was higher for the hens on a high target BW treatment compared to a standard target BW treatment at the end of wk 55 (129.4 vs. 92.8, respectively; van

der Klein et al., 2018). The onset of sexual maturity can be affected by the concentration of reproductive hormones. The peak concentration of E2 for broiler breeder hens provided either restricted or *ad libitum* access to feed occurred between 5.69 and 6.63 d prior to sexual maturity, respectively (Renema et al., 1999b). Interestingly, the concentration of plasma LH and FSH increased in *ad libitum* fed broiler breeder hens relative to restricted birds and indicated that energy balance may interact with BW and age threshold (HPG axis maturation) to initiate sexual maturity (Renema et al., 1999b). In the current study, in the High MEI treatment compared to the Low MEI treatment, the plasma LH was 1.9 times higher from 22 to 26 wk of age. Moreover, in the High MEI treatment compared to the Low MEI treatment, the plasma FSH and E2 were both 1.6 times higher from 22 to 26 wk of age. The increased reproductive hormones indicated more robust ovary development in the High MEI pullets compared to the Low MEI pullets. In addition, in the High MEI treatment compared to the Low MEI treatment, the GnRH-I mRNA level was 2.3 fold higher in the hypothalamus and the GnRH-RI mRNA level was 1.8 fold higher in the pituitary from 22 to 26 wk of age. These results indicated that pullets in the High MEI treatment were better prepared to respond to photostimulation with a more responsive HPG axis leading to advance onset of lay compared to the Low MEI pullets. In addition, these results are consistent with the hypothesis that current broiler breeder BW recommendations may restrict pullets from entering lay since the proportional degree of feed restriction is increasing whereas little change has occurred to broiler breeder BW recommendations (Zuidhof, 2018). Therefore, the results of the current experiment would suggest

increasing the target BW for broiler breeder pullets to help them to obtain all the cascades for the sexual maturity earlier and advance the onset of lay.

5.5 Conclusion

Higher ME intake of pullets in the High MEI treatment resulted in greater BW compared to the pullets in the Low MEI treatment. Lipid content was also higher in the High MEI treatment than the Low MEI treatment from 23 to 26 wk of age, which resulted in pullets in the High MEI treatment having more energy available to trigger the onset of sexual maturity. Pullets in the High MEI treatment increased the mRNA level of GnRH-I in the hypothalamus and the mRNA level of its receptor (GnRH-RI) in the pituitary relative to the pullets in the Low MEI treatment. The ratio of GnRH-RI to GnIH-R was 1.38 times higher for the pullets in the High MEI pullets than the pullets in the Low MEI treatment. The ratio of GnRH-RI to GnRH-RIII was 1.63 times higher for the pullets in the High MEI treatment than the pullets in the Low MEI treatment. Moreover, in the hypothalamus, the mRNA level of GnIH decreased with age while the pituitary mRNA level of LH increased with age. After photostimulation, higher ME intake increased GnRH-I and GnRH-RI mRNA levels leading to the increase in LH mRNA levels. It seems that high ME intake along with photostimulation can increase GnRH-RI expression. Plasma levels of LH, FSH, and E2 were higher in the High MEI treatment than the Low MEI treatment from 22 to 26 wk of age, consistent with advanced ovary development in the High MEI treatment than the Low MEI treatment. Therefore, the higher ME intake advanced the activation of the HPG axis and increased body lipid deposition, and stimulated reproductive

hormone levels which overall accelerated puberty in broiler breeder pullets. Among the anorexigenic and the orexigenic genes that were assessed in the current study, the mRNA levels of POMC were increased in the High MEI treatment than the Low MEI treatment from 22 to 26 wk of age. Down-regulation of the POMC gene in the Low MEI pullets may have occurred via AMPK activation.

5.6 Acknowledgments

Financial support from Alberta Agriculture and Forestry (Edmonton, Alberta, Canada), Alberta Innovates Bio Solutions (Edmonton, Alberta, Canada), Agriculture and Food Council (Edmonton, Alberta, Canada), The Alberta Chicken Producers (Edmonton, Alberta, Canada), Poultry Industry Council (Guelph, Ontario, Canada), Danisco Animal Nutrition (DuPont; Marlborough, Wiltshire, United Kingdom), The Canadian Hatching Egg Producers (Ottawa, Ontario, Canada), The Alberta Hatching Egg Producers (Edmonton, Alberta, Canada), and The Ontario Broiler Chicken Hatching Egg Producers Association (Guelph, Ontario, Canada) is gratefully acknowledged. In kind technical support was provided by Xanantec Technologies, Inc. (Edmonton, Alberta, Canada), excellent technical expertise provided by staff and students at the University of Alberta Poultry Unit (Edmonton, Alberta, Canada), and base supporters of the Poultry Research Centre (Edmonton, Alberta, Canada) are also gratefully acknowledged.

5.7 References

Andersen, C. L., J. L. Jensen, and T. F. Ørntoft. 2004. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation

approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer. Res. 64:5245–5250.

AOAC. 2007. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Aviagen, 2011. Ross 308 Parent Stock: Performance Objectives. Aviagen Inc., Huntsville, AL. Accessed Apr. 2015. http://en.aviagen.com/assets/Tech_Center/Ross_PS/Ross-308-PS-PO2011.pdf.

Aviagen. 2013. Ross 308 PS nutrition specifications. Aviagen Incorporated, USA. http://sedghi.iut.ac.ir/sites/sedghi.iut.ac.ir/files//files_course/ross308ps-nutritionspecs-2013final.pdf. Accessed November 2016.

Baxter, M., N. Joseph, V. R. Osborne, and G. Y. Bedecarrats. 2014. Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. Poult. Sci. 93:1289–1297.

Bédécarrats, G.Y., M. Shimizu, and D. Guemene. 2006. Gonadotropin releasing hormones and their receptors in avian species. J. Poult. Sci. 43:199–214.

Bédécarrats**,** G. Y., H. McFarlane, S. R. Maddineni, and R. Ramachandran. 2009. Gonadotropin-inhibitory hormone receptor signaling and its impact on reproduction in chickens. Gen. Comp. Endocrinol. 163:7–11.

Bédécarrats**,** G. Y, M. Baxter, and B. Sparling. 2016. An updated model to describe the neuroendocrine control of reproduction in chickens. Gen. Comp. Endocrinol. 227:58–63.

Bédécarrats**,** G. Y. 2015. Control of the reproductive axis: Balancing act between stimulatory and inhibitory inputs. Poult. Sci. 94:810–815.

Bentley, G. E., J. P. Jensen, G. J. Kaur, D. W. Wacker, K. Tsutsui, J. C. Wingfield. 2006. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). Horm. Behav. 49:550–555.

Bentley, G. E., N. Perfito, K. Ukena, K. Tsutsui, and J. C. Wingfield. 2003. Gonadotropin-inhibitory peptide in song sparrows (*Melospiza melodia*) in different reproductive conditions, and in house sparrows (*Passer domesticus*) relative to chicken-gonadotropin-releasing hormone. J. Neuroendocrinol. 15:794–802.

Bentley, G. E., T. Ubuka, N. L. McGuire, V. S. Chowdhury, Y. Morita, T. Yano, I. Hasunuma, M. Binns, J. C. Wingfield, K. Tsutsui. 2008. Gonadotropininhibitory hormone and its receptor in the avian reproductive system. Gen. Comp. Endocrinol. 156:34–43.

Carling, D. 2004. The AMP-activated protein kinase cascade—A unifying system for energy control. Trends. Biochem. Sci. 29:18–24.

Chen, G., F. Yang, T. Wu, J. Jiang, and W. Zhou. 2016. The stimulatory effect of cerebral intraventricular injection of cNPY on precocial feeding behavior in neonatal chicks (*Gallus domesticus*). PLoS. One. 11:e0153342.

Cheung, C. C., J. E. Thornton, J. L. Kuijper, D. S. Weigle, D. K. Clifton, and R. A. Steiner. 1997. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinol. 138: 855–858.

Clarke, I. J., and J. T. Cummins. 1984. Direct pituitary effects of estrogen and progesterone on gonadotropin secretion in the ovariectomized ewe. Neuroendocrinol. 39:267–274.

Couse, J. F., and K. S. Korach. 1999. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. 20:358–417.

Denbow, D. M., S. Meade, A. Robertson, J. P. McMurtry, M. Richards, and C. Ashwell. 2000. Leptin-induced decrease in food intake in chickens. Physiol. Behav. 69:359–362.

Eitan, Y., E. Lipkin, and M. Soller. 2014. Body composition and reproductive performance at entry into lay of anno 1980 versus anno 2000 broiler breeder females under fast and slow release from feed restriction. Poult. Sci. 93:1227–1235.

Elias, C. F., C. Asckenasi, C. Lee, J. Kelly, R. S. Ahima, C. Bjørbæk, J. K. Flier, C. B. Saper, and J. K. Elmquisk. 1999. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron. 23:775–786.

Etches, R. J., and C. E. Duke. 1984. Progesterone, androstenedione and oestradiol content of the theca and granulosa tissues of the four largest ovarian follicles during the ovulatory cycle of the hen (*Gallus domesticus*). J. Endocrinol. 103:71–76.

Friedman, J. M., and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature. 395:763–770.

Friedman-Einat M., L. A. Cogburn, S. Yosefi, G. Hen, D. Shinder, A. Shirak, and et al. 2014. Discovery and characterization of the first genuine avian leptin gene in the rock dove (*Columba livia*). Endocrinol. 155:3376–84.

Furuse, M. 2002. Central regulation of food intake in the neonatal chick. Anim. Sci. J. 73:83–94.

Furuse, M., H. Yamane, S. Tomonaga, Y. Tsuneyoshi, and D. M. Denbow. 2007. Neuropeptidergic regulation of food intake in the neonatal chick: A review. Jpn. Poult. Sci. 44:349–356.

Hadinia, S. H., P. R. O. Carneiro, C. A. Ouellette, and M. J. Zuidhof. 2018. Energy partitioning by broiler breeder pullets in skip-a-day and precision feeding systems. 97:4279–4289.

Halaas, J. L., C. Boozer, J. Blair-West, N. Fidahusein, D. A. Denton, J. M. Friedman. 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc. Natl. Acad. Sci. USA. 94:8878–8883.

Horev, G., P. Einat, T. Aharoni, Y. Eshdat, and M. Friedman-Einat. 2000. Molecular cloning and properties of the chicken leptin-receptor (CLEPR) gene. Mol. Cell. Endocrinol. 162:95–106.

Imai, K., and A. V. Nalbandov. 1978. Plasma and follicular steroid levels of laying hens after the administration of gonadotropins. Biol. Reprod. 19:779–784.

Johnston, D. W. 1970. Caloric density of avian adipose tissue. Comp. Biochem. Physiol. 34:827–832.

Joseph, N., K. Morgan, R. Sellar, D. McBride, R. P. Millar, and I. C. Dunn. 2009. J. Endocrinol. 202:179–190.

Katz, I. A., R. P. Millar, and J. A. King. 1990. Differential regional distribution and release of two forms of gonadotropin releasing hormone in the chicken brain. Peptides. 11:443–450.

Kuenzel, W. J., L. W. Douglass, and B. A. Davison. 1987. Robust feeding following central administration of neuropeptide Y or peptide YY in chicks, *Gallus domesticus*. Peptides. 8:823–828.

Kuenzel, W. J. 1994. Central neuroanatomical systems involved in the regulation of food intake in birds and mammals. J. Nutr. 124:1355S–1370S.

Kuenzel, W. J., M. M. Beck, and R. Teruyama. 1999. Neural sites and pathways regulating food intake in birds: A comparative analysis to mammalian systems. J. Exp. Zool. 283:348–364.

Leffler, T. P., C. R. Moser, B. J. Mcmanus, and J. J. Urh. 2008. Determination of Moisture and Fat in Meats by Microwave and Nuclear Magnetic Resonance Analysis: Collaborative Study. J. AOAC. Int. 91:802–810.

Liu, L., D. Li, E. R. Gilbert, Q. Xiao, X. Zhao, Y. Wang, H. Yin, Q. Zhu. 2015. Effect of monochromatic light on expression of estrogen receptor (ER) and progesterone receptor (PR) in ovarian follicles of chicken. PLoS ONE. 10:e0144102.

Long, Y. C., and J. R. Zierath. 2006. AMP-activated protein kinase signaling in metabolic regulation. J. Clin. Invest. 116:1776–1783.

McLean, B.K., N. Chang, and M.B. Nikitovitch-Winer. 1975. Ovarian steroids directly alter luteinizing hormone (LH) release by pituitary homografts. Endocrinol. 97:196–201.

Maddineni, S., O. M. Ocon-Grove, S. M. Krzysik-Walker, G. L. 3rd Hendricks, J. A. Proudman, and R. Ramachandran. 2008. Gonadotrophin-inhibitory hormone receptor expression in the chicken pituitary gland: Potential influence of sexual maturation and ovarian steroids. J. Neuroendocrinol. 20:1078–1088.

Minokoshi, Y., T. Alquier, N. Furukawa, Y. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferre´, M. J. Birnbaum, B. J. Stuck, and B. B. Kahn. 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature. 428:569–574.

Miyamoto, K., Y. Hasegawa, M. Igarashi, N. Chino, S. Sakakibara, K. Kangawa, and H. Matsuo. 1983. Evidence that chicken hypothalamic luteinizing hormone-releasing hormone is [Gln2]-LH-RH. Life Sci. 32:1341–1347.

Miyamoto, K., Y. Hasegawa, T. Minegishi, M. Nomura, Y. Takahashi, M. Igarashi, K. Kangawa, and H. Matsuo. 1982. Isolation and characterization of chicken hypothalamic luteinizing hormone-releasing hormone. Biochem. Biophys. Res. Commun. 107:820–827.

Ohkubo, T., M. Tanaka, and K. Nakashima. 2000. Structure and tissue distribution of chicken leptin receptor (cOb-R) mRNA. Biochim. Biophys. Acta. 1491:303–308.

Onagbesan, O. M., S. Metayer, K. Tona, J. Williams, E. Decuypere, and V. Bruggeman. 2006. Effects of genotype and feed allowance on plasma luteinizing hormones, follicle-stimulating hormones, progesterone, estradiol levels, follicle differentiation, and egg production rates of broiler breeder hens. Poult. Sci. 85:1245–1258.

Paczoska-Eliasiewicz, H. E., A. Gertler, M. Proszkowiec, J. Proudman, A. Hrabia, A. Sechman, M. Mika, T. Jacek, S. Cassy, N. Raver, and J. Rzasa. 2003. Attenuation by leptin of the effects of fasting on ovarian function in hens (*Gallus demosticus*). Reproduction. 126:739–751.

Pearson, R. A., and K. M. Herron. 1981. Effects of energy and protein allowances during lay on the reproductive performance of broiler breeder hens. Br. Poult. Sci. 22:227–239.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucl. Acids Res. 29:e45.

Prokop, J. W., C. Schmidt, D. Gasper, R. J. Duff, A. Milsted, T. Ohkubo, and et al. 2014. Discovery of the elusive leptin in birds: identification of several "missing links" in the evolution of leptin and its receptor. PLoS. One. 9:e92751.

Proszkowiec-Weglarz, M., M. P. Richards, and J. P. McMurtry. 2006a. Molecular cloning, genomic organization and expression of three chicken 5′-AMP-activated protein kinase gamma subunit genes. Poult. Sci. 85:2031–2041.

Proszkowiec-Weglarz, M., M. P. Richards, R. Ramachandran, and J. P. McMurtry. 2006b. Characterization of the AMP-activated protein kinase pathway in chickens. Comp. Biochem. Physiol. B. 143:92–106.

Proszkowiec-Weglarz, M., and M. P. Richards. 2007. 5′-AMP-Activated protein kinase in avian biology. Avian. Poult. Biol. Rev. 18:123–145.

Pullar, J. D., and A. J. F. Webster. 1977. The energy cost of protein and fat deposition in the rat. Br. J. Nutr. 37:355–361.

Renema, R. A., M. E. Rustad, and F. E. Robinson. 2007. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. World's Poult. Sci. J. 63:457–472.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I. McKay. 1999a. Effects of BW and feed allocation during sexual maturation in broiler breeder hens. 1. Growth and carcass characteristics. Poult. Sci. 78:629–639.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I. McKay. 1999b. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 2. Ovarian morphology and plasma hormone profiles. Poult. Sci. 78:629–639.

Richards, M. P. 2003. Genetic regulation of feed intake and energy balance. Poult. Sci. 82:907–916.

Richards, M. P., and M. Proszkowiec-Weglarz. 2007. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poult. Sci. 86:1478–1490.

Richards, M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMurtry. 2010. Feed intake regulation for the female broiler breeder: In theory and in practice. J. Appl. Poult. Res. 19:182–193.

Robinson, F. E., and R. J. Etches. 1986. Ovarian steroidogenesis during follicular maturation in the domestic fowl (*Gallus domesticus*). Biol. Reprod. 35:1096–1105.

Robinson, F. E., G. M. Fasenko, R. A. Renema. 2003. Female reproduction: control of ovarian function. Pages 3-10 in Optimizing Chick Production in Broiler

Breeders. F. E. Robinson, and R. A. Renema, ed. Spotted Cow Press, Edmonton, Canada.

Scott, T. A., and F. Boldaji. 1997. Comparison of inert markers [chromic oxide or insoluble ash (Celite)] for determining apparent metabolizable energy of wheat- or barley-based broiler diets with or without enzymes. Poult. Sci. 76:594–598.

Seroussi, E., Y. Cinnamon, S. Yosefi, O. Genin, J. G. Smith, N. Rafati, and et al. 2016. Identification of the long-sought leptin in chicken and duck: expression pattern of the highly GC-rich avian leptin fits an autocrine/paracrine rather than endocrine function. Endocrinol. 157:737–751.

Shaw, N. D., S. N. Histed, S. S. Srouji, J. Yang, H. Lee, and J. E. Hall. 2010. Estrogen negative feedback on gonadotropin secretion: evidence for a direct pituitary effect in women. J. Clin. Endocrinol. Metab. 95:1955–1961.

Shimizu, M., and G. Y. Bédécarrats. 2010. Activation of the chicken gonadotropin-inhibitory hormone receptor reduces gonadotropin releasing hormone receptor signaling. Gen. Comp. Endocrinol. 167:331–337.

Shimizu, M., and G. Y. Bédécarrats. 2006. Identification of a novel pituitary-specific chicken gonadotropin-releasing hormone receptor and its splice variants. Biol. Reprod. 75:800–808.

Summers, J. D., and S. Leeson. 1983. Factors influencing early egg size. Poult. Sci. 62:1155–1159.

Sun, Y. M., I. C. Dunn, E. Baines, R. T. Talbot, N. Illing, R. P. Millar, and P. J. Sharp. 2001. Distribution and regulation by oestrogen of fully processed and variant transcripts of gonadotropin releasing hormone I and gonadotropin

releasing hormone receptor mRNAs in the male chicken. J. Neuroendocrinol. 13:37–49.

Tsutsui, K., G. E. Bentley, G. Bedecarrats, T. Osugi, T. Ubuka, and L. J. Kriegsfeld. 2010a. Gonadotropin-inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. Front. Neuroendocrinol. 31:284–295.

Tsutsui, K., G. E. Bentley, L. J. Kriegsfeld, T. Osugi, J. Y. Seong, H. Vaudry. 2010b. Discovery and evolutionary history of gonadotrophin-inhibitory hormone and kisspeptin: new key neuropeptides controlling reproduction. J. Neuroendocrinol. 22:716–727.

Tsutsui, K. 2009. A new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH): Biosynthesis, mode of action and functional significance. Prog. Neurobiol. 88:76–88.

Tsutsui, K., E. Saigoh, K. Ukena, H. Teranishi, Y. Fujisawa, M. Kikuchi, S. Ishii, and P. J. Sharp. 2000. A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem. Biophys. Res. Commun. 275:661–667.

Ubuka, T., G. E. Bentley, K. Ukena, J. C. Wingfield, and K. Tsutsui. 2005. Melatonin induces the expression of gonadotropin-inhibitory hormone in the avian brain. Proc. Natl. Acad. Sci. U. S. A. 102:3052–3057.

van der Klein, S. A. S., G. Y. Bédécarrats, F. E. Robinson, and M. J. Zuidhof. 2018. Early photostimulation at the recommended body weight reduced broiler breeder performance. Poult. Sci. 97:3736–3745.

van Emous, R. 2015. Body composition and reproduction in broiler breeders: impact of feeding strategies. PhD Diss. Univ. Wageningen, Wageningen, the Netherland.

Vanmontfort, D., L. R. Berghman, L. Rombauts, G. Verhoeven, and E. Decuypere. 1995. Developmental changes in immunoreactive inhibin and FSH in plasma of chickens from hatch to sexual maturity. Br. Poult. Sci. 36:779–790.

Wang, D., C. Xu, T. Wang, H. Li, Y. Li, J. Ren, and et al. 2016. Discovery and functional characterization of leptin and its receptors in Japanese quail (*Coturnix japonica*). Gen. Comp. Endocrinol. 225:1–12.

Welt, C. K, Y. L. Pagan, P. C. Smith, K. B. Rado, and J. E. Hall. 2003. Control of follicle-stimulating hormone by estradiol and the inhibins: critical role of estradiol at the hypothalamus during the luteal-follicular transition. J. Clin. Endocrinol. Metab. 88:1766–1771.

Winder, W. W., and D. M. Thompson. 2007. Cellular energy sensing and signaling by AMP-activated protein kinase. Cell. Biochem. Biophys. 47:332–347.

Zuidhof, M. J. 2018. Lifetime productivity of conventionally and precision-fed broiler breeders. Poult. Sci. 97:3921–3937.

Zuidhof, M. J., M. V. Fedorak, C. C. Kirchen, E. H. M. Lou, C. A. Ouellette, and I. I. Wenger. 2016. System and method for feeding animals. PrecisionZX, Inc., assignee. United States Patent Application No. 15/283,125.

Zuidhof, M. J., M. V. Fedorak, C. A. Ouellette, and I. I. Wenger. 2017. Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity. Poult. Sci. 96:2254–2263.

5.8 Tables

Table 5-1. Composition of broiler breeder pullet grower diets for low ME intake (Low MEI) and high ME intake (High MEI) treatments

Ingredient	Low MEI	High MEI
	g/kg	
Corn	327.6	312.0
Wheat	343.0	326.4
Soybean meal	65.3	62.1
Oats	60.7	57.1
Canola meal	78.4	74.6
Wheat Bran	49.0	46.6
Canola oil	9.8	57.0
Ground limestone	14.9	14.1
Dicalcium phosphate	14.7	14.0
Choline chloride premix	4.9	4.7
Vitamin premix[1]	2.5	2.5
Mineral premix[2]	2.5	2.5
NaCl	3.8	3.7
D, L-methionine	0.9	0.9
L-lysine	1.2	1.1
Threonine	0.3	0.2
Enzyme[3]	0.5	0.5
Celite[4]	20	20
Total:	1,000	1,000
Analyzed composition, as fed basis		
AME (kcal/kg)	2,807	3,109
CP (g/kg)[5]	147	143
Calculated composition, as fed basis		
AME (kcal/kg)	2,696	2,931
CP (g/kg)	154	152
Calcium (g/kg)	10	10
Non-phytate phosphorous (g/kg)	4.5	4.3
Available lysine (g/kg)	7.4	7.1
Available methionine (g/kg)	3.4	3.2
Available methionine + cysteine (g/kg)	6.3	6.3

[1] Premix provided per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; cholecalciferol, 4,000 IU; Vitamin E (DL- α-tocopheryl acetate), 50.0 IU; vitamin K, 4.00 mg; pantothenic acid, 15.0 mg; riboflavin, 10.0 mg; folacin, 2.00 mg; niacin, 65 mg; thiamine, 4.00 mg; pyridoxine, 5.00 mg; vitamin B12, 0.02 mg; biotin, 0.20 mg

[2] Premix provided per kilogram of diet: iodine, 1.65 mg; Mn, 120 mg; Cu, 20.0 mg; Zn, 100 mg; Se, 0.30 mg; Fe, 80.0 mg

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

[4] Acid-insoluble ash marker (Celite 281, Lompoc, CA) was added to determine the AME content of the diets

[5] Analyzed N using Leco TruMac (Leco Corporation, St. Joseph, Michigan, USA)

Table 5-2. Sequences of the oligonucleotide primers used in quantitative real-time PCR, and the publicly available sequences they were designed from

	Primer sequence (5'- 3')			
Gene*	Forward	Reverse	Size for PCR product (bp)	GenBank Accession no.
ACTB	TGTTACCAACACCCACACCC	TCCTGAGTCAAGCGCCAAAA	110	NM_205518
EEF1A1	CTCTCACCTGGAACCAACTATTC	CCACTGTTTGGCATTGGTATTG	100	NM_204157.2
SDHA	GACAGAGGCATTGTGTGGAA	CGAGCCTCAGCACCATAAAT	98	NM_001277398.1
GnRH-I	CACCCAGCTGCTCCAATTA	CAGGTAATGCCACCTCATTCT	100	X69491.1
GnIH	GGAAGTCAGTGCCCATCAATC	ACGCTGCATCTTTTCCGAGT	130	NM_204363.1
GnRH-RI	GGGAGATCAGTAAGCAGCTAAAG	GCTGGCAACAATCACAATGG	113	NM_204653.1, XM_015292070
GnRH-RIII	ATGTACGCCTCCGCCTTCGT	GCAGGGTGACGGTGTGGAAG	178	AY895154
GnIH-R	GCATGTCTGTCTCCGCCTCT	GTGGACGATGCAGCGAAACC	71	AB120326
LH	TCGCCCCATAAACGTAACGG	CGTGGTGGTCACAGCCATAC	70	HQ872606.1
FSH	CCACGTGGTGCTCAGGATACT	AGGTACATATTTGCTGAACAGATGAGA	84	NM_204257.1
NPY	GAGGCACTACATCAACCTCATC	TCTGTGCTTTCCCTCAACAA	96	M87294.1
POMC	AGGAGACCCATCAAGGTGTA	TTCCTCCTCTTCTTCCTCCTC	135	NM_001031098.1
LEPR	ACCGAAGAATGAAGAAACTGCT	TGACAAAAAGGTGCTCAAAAGT	111	NM_204323.1

*Actin beta (ACTB), Eukaryotic translation elongation factor 1 alpha 1 (EEF1A1), Succinate dehydrogenase complex flavoprotein subunit A (SDHA), Gonadotropin releasing hormone-I (GnRH-I), Gonadotropin inhibitory hormone (GnIH), Gonadotopin releasing hormone receptor-I (GnRH-RI), Gonadotropin releasing hormone receptor- III (GnRH-RIII), Gonadotropin inhibitory hormone receptor (GnIH-R), Luteinizing hormone beta subunit (LH), Follicle stimulating hormone beta subunit (FSH), Neuropeptide Y (NPY), Proopiomelanocortin (POMC), Leptin receptor (LEPR).

Table 5-3. Body weight (BW)[1], ME intake (MEI), and percentage of flock in lay for broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 28 wk of age

Treatment	Age (wk)	Age (d)	BW	SEM	Age (wk)	MEI	SEM	Hens in lay	SEM
			g			kcal/d		%	
Low MEI			2,943[s]	5		258[s]	3.8	32.1[s]	3.6
High MEI			3,569[r]	8		489[r]	6.4	80.1[r]	3.0
	22	160	2,738[e]	6	21 to 22	362	4.5	-	-
	23	167	3,032[d]	6	22 to 23	373	4.8	-	-
	24	174	3,340[c]	7	23 to 24	367	5.2	13.7[d]	3.8
	25	181	3,547[b]	9	24 to 25	378	6.8	48.3[c]	3.8
	26	188	3,623[a]	19	25 to 26	386	14.8	65.7[b]	3.8
	27	195	-	-	26 to 27	-	-	73.5[a]	3.8
	28	202	-	-	27 to 28	-	-	79.7[a]	3.8
Low MEI	22	160	2,641[o]	8	21 to 22	266[j]	6.3	-	-
	23	167	2,789[n]	9	22 to 23	243[k]	6.7	-	-
	24	174	2,928[l]	9	23 to 24	228[k]	7.3	6.6[m]	4.6
	25	181	3,095[k]	11	24 to 25	271[j]	8.8	20.2[l]	4.6
	26	188	3,263[j]	16	25 to 26	279[j]	12.1	30.0[l]	4.6
	27	195	-	-	26 to 27	-	-	45.6[k]	4.6
	28	202	-	-	27 to 28	-	-	58.1[j]	4.6
High MEI	22	160	2,834[m]	8	21 to 22	458[i]	6.4	-	-
	23	167	3,276[j]	9	22 to 23	502[h]	6.8	-	-
	24	174	3,751[i]	10	23 to 24	506[h]	7.4	20.8[l]	4.2
	25	181	4,000[h]	13	24 to 25	486[h]	10.4	76.6[i]	4.2
	26	188	3,983[h]	34	25 to 26	493[hi]	27.1	101[h]	4.2
	27	195	-	-	26 to 27	-	-	101[h]	4.2
	28	202	-	-	27 to 28	-	-	101[h]	4.2
Source of variation			P-value			P-value		P-value	
Treatment			< 0.001			< 0.001		< 0.001	
Age			< 0.001			0.16		< 0.001	
Treatment x Age			< 0.001			< 0.001		< 0.001	

[1]At 21 wk of age (153 d), before starting the current experiment the BW of pullets in the High MEI and in the Low MEI treatments did not differ (2,489 ± 7 and 2,484 ±7 g respectively; P > 0.05).

[r-s] means within column within treatment with no common superscript differ (P < 0.05)

[a-e] means within column within age with no common superscript differ (P < 0.05)

[h-o] means within column within treatment x age with no common superscript differ (P < 0.05)

Table 5-4. Relative expression[1] of gonadotropin releasing hormone (GnRH-I) and gonadotropin inhibitory hormone (GnIH) in hypothalamus and GnRH-I receptor-I (GnRH-RI), GnRH-I receptor-III (GnRH-RIII), GnIH receptor (GnIH-R) genes in anterior pituitary of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age

Treatment	Age (wk)	GnRH-I	SEM	GnIH	SEM	GnRH-RI	SEM	GnRH-RIII	SEM	GnIH-R	SEM
		Relative expression									
Low MEI		0.32^{s}	0.12	1.50	0.16	1.54^{s}	0.29	1.25	0.13	3.51	1.48
High MEI		0.74^{r}	0.13	1.63	0.17	2.84^{r}	0.30	1.17	0.14	2.27	1.56
	22	0.51^{b}	0.26	1.43^{ab}	0.34	1.65^{bc}	0.57	0.87	0.26	1.71	2.93
	23	1.20^{a}	0.23	2.12^{a}	0.30	1.35^{bc}	0.57	1.08	0.26	1.67	2.93
	24	0.40^{b}	0.15	2.09^{a}	0.20	1.62^{c}	0.38	0.99	0.17	3.29	1.96
	25	0.38^{b}	0.15	1.36^{b}	0.20	2.72^{ab}	0.38	1.51	0.17	2.73	1.96
	26	0.16^{b}	0.22	0.81^{b}	0.26	3.59^{a}	0.49	1.58	0.22	5.04	2.54
Low MEI	22	0.34^{i}	0.36	0.98	0.48	1.06^{j}	0.78	0.91	0.35	2.20	4.02
	23	0.63^{i}	0.31	1.89	0.42	1.44^{j}	0.78	1.08	0.35	1.68	4.02
	24	0.25^{i}	0.22	1.95	0.29	1.59^{j}	0.54	1.21	0.24	4.91	2.77
	25	0.14^{i}	0.22	1.25	0.29	1.61^{j}	0.54	1.38	0.24	1.02	2.77
	26	0.24^{i}	0.22	1.16	0.29	1.98^{j}	0.54	1.65	0.24	7.73	2.77
High MEI	22	0.68^{i}	0.36	1.88	0.48	2.24^{ij}	0.78	0.83	0.35	1.22	4.02
	23	1.77^{h}	0.31	2.36	0.42	1.26^{j}	0.78	1.09	0.35	1.66	4.02
	24	0.55^{i}	0.22	2.24	0.29	1.66^{j}	0.54	0.77	0.24	1.67	2.77
	25	0.62^{i}	0.22	1.48	0.29	3.84^{hi}	0.54	1.65	0.24	4.44	2.77
	26	0.09^{i}	0.36	0.47	0.43	5.20^{h}	0.81	1.52	0.37	2.35	4.17
Egg*(covariate)		0.34		0.48		-0.59		0.38		0.35	
Source of variation		P-value									
Egg		0.081		0.070		0.22		0.080		0.89	
Treatment		0.021		0.59		0.003		0.70		0.57	
Age		0.031		0.002		0.010		0.090		0.91	
Treatment x Age		0.32		0.28		0.050		0.70		0.66	

[r-s] means within column within treatment with no common superscript differ ($P < 0.05$)

[a-c] means within column within age with no common superscript differ ($P < 0.05$)

[h-j] means within column within treatment x age with no common superscript differ ($P < 0.05$)

[*]The covariate egg was 0 for birds that had not laid an egg and was 1 for birds that had laid an egg.

[1]Relative expression was based on the expression levels of a target gene versus housekeeping genes. Actin beta and succinate dehydrogenase complex flavoprotein subunit A were selected as housekeeping genes to normalize expression measured in hypothalamus tissues and actin beta and eukaryotic translation elongation factor 1 alpha 1 were selected to normalize expression measured in anterior pituitary tissues.

Table 5-5. Relative expression[1] of luteinizing hormone (LH) and follicular stimulating hormone (FSH) genes in anterior pituitary and neuropeptide Y (NPY), proopiomelanocortin (POMC), and leptin receptor (LEPR), genes in hypothalamus of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age

Treatment	Age (wk)	LH	SEM	FSH	SEM	NPY	SEM	POMC	SEM	LEPR	SEM
		Relative expression									
Low MEI		0.63	0.12	0.58	0.08	1.93	0.24	0.94^{s}	0.13	1.26	0.08
High MEI		0.80	0.13	0.63	0.09	2.46	0.25	1.47^{r}	0.13	1.43	0.08
	22	0.06^{c}	0.24	0.76^{ab}	0.16	1.50	0.51	1.49^{a}	0.26	1.06	0.17
	23	0.04^{c}	0.24	1.05^{a}	0.16	2.50	0.45	1.98^{a}	0.25	1.64	0.15
	24	0.16^{c}	0.16	0.49^{bc}	0.11	1.92	0.30	1.54^{a}	0.15	1.34	0.10
	25	1.12^{b}	0.16	0.33^{c}	0.11	2.38	0.30	0.74^{b}	0.15	1.30	0.10
	26	2.21^{a}	0.21	0.40^{bc}	0.14	2.67	0.39	0.28^{b}	0.20	1.37	0.13
Low MEI	22	0.06	0.33	0.74	0.22	0.89	0.71	0.98^{jklm}	0.36	0.98	0.23
	23	0.05	0.33	1.16	0.22	1.81	0.62	1.22^{ijkl}	0.36	1.49	0.20
	24	0.16	0.22	0.33	0.15	1.88	0.42	1.48^{ijk}	0.22	1.24	0.14
	25	0.85	0.22	0.30	0.15	2.18	0.42	0.60^{lm}	0.22	1.25	0.14
	26	2.04	0.22	0.36	0.15	2.88	0.42	0.45^{lm}	0.22	1.34	0.14
High MEI	22	0.07	0.33	0.78	0.22	2.10	0.71	2.01^{hi}	0.36	1.14	0.23
	23	0.02	0.33	0.95	0.22	3.19	0.62	2.75^{h}	0.32	1.78	0.20
	24	0.16	0.22	0.64	0.15	1.97	0.42	1.61^{ij}	0.22	1.45	0.14
	25	1.38	0.22	0.35	0.15	2.58	0.42	0.87^{klm}	0.22	1.36	0.14
	26	2.38	0.34	0.44	0.23	2.47	0.64	0.11^{m}	0.32	1.41	0.21
Egg*(covariate)		-1.03		0.08		0.09		0.23		-0.08	
Source of variation		P-value									
Egg		< 0.001		0.56		0.80		0.24		0.51	
Treatment		0.33		0.67		0.13		0.005		0.14	
Age		< 0.001		0.010		0.29		< 0.001		0.11	
Treatment x Age		0.73		0.72		0.49		0.030		0.97	

[r-s] means within column within treatment with no common superscript differ ($P < 0.05$)

[a-c] means within column within age with no common superscript differ ($P < 0.05$)

[h-m] means within column within treatment x age with no common superscript differ ($P < 0.05$)

[*]The covariate egg was 0 for birds that had not laid an egg and was 1 for birds that had laid an egg

[1]Relative expression was based on the expression levels of a target gene versus housekeeping genes. Actin beta and succinate dehydrogenase complex flavoprotein subunit A were selected as housekeeping genes to normalize expression measured in hypothalamus tissues and actin beta and eukaryotic translation elongation factor 1 alpha 1 were selected to normalize expression measured in anterior pituitary tissues

Table 5-6. The ratios of relative expression of gonadotropin releasing hormone (GnRH-I) receptor-I (GnRH-RI), GnRH-I receptor-III (GnRH-RIII), and gonadotropin inhibitory hormone receptor (GnIH-R) genes in anterior pituitary of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age

Treatment	Age (wk)	GnRH-RI:GnRH-RIII ratio	SEM	GnRH-RI:GnIH-R ratio	SEM	GnRH-RIII:GnIH-R ratio	SEM
Low MEI		1.69[s]	0.26	1.06[s]	0.14	0.89	0.10
High MEI		2.75[r]	0.28	1.46[r]	0.14	0.63	0.10
	22	2.85	0.52	1.54	0.27	0.6791	0.19
	23	1.78	0.52	1.17	0.27	0.7030	0.19
	24	1.83	0.35	0.85	0.18	0.7044	0.13
	25	1.72	0.35	1.18	0.19	1.0279	0.13
	26	2.89	0.45	1.56	0.24	0.6939	0.17
Low MEI	22	2.15	0.72	0.84[j]	0.37	0.67	0.26
	23	1.81	0.72	1.18[ij]	0.37	0.70	0.26
	24	2.17	0.49	0.72[j]	0.26	0.52	0.18
	25	2.27	0.49	1.45[hij]	0.27	0.65	0.18
	26	3.94	0.74	1.12[ij]	0.26	0.60	0.27
High MEI	22	3.55	0.72	2.25[h]	0.37	0.69	0.26
	23	1.81	0.72	1.16[ij]	0.37	0.70	0.26
	24	2.17	0.49	0.99[j]	0.26	0.52	0.18
	25	2.27	0.49	0.92[j]	0.26	0.65	0.18
	26	3.94	0.74	2.00[hi]	0.39	0.60	0.27
Egg* (covariate)		-0.46	0.44	0.02	0.23	0.12	0.16
Source of variation		P-value					
Egg		0.30		0.93		0.45	
Treatment		0.008		0.049		0.071	
Age		0.11		0.10		0.32	
Treatment x Age		0.58		0.030		0.32	

[r-s] means within column within treatment with no common superscript differ ($P < 0.05$)

[a-b] means within column within age with no common superscript differ ($P < 0.05$)

[h-j] means within column within treatment x age with no common superscript differ ($P < 0.05$)

*The covariate egg was 0 for birds that had not laid an egg and was 1 for birds that had laid an egg

Table 5-7. Plasma concentrations of luteinizing hormone (LH), follicular stimulating hormone (FSH), and 17 beta-estradiol (E2) of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age

Treatment	Age (wk)	LH	SEM	FSH	SEM	E2	SEM
		ng/mL		pg/mL		pg/mL	
Low MEI		1.60[s]	0.30	89.3[s]	13.6	266[s]	47.5
High MEI		3.05[r]	0.31	145[r]	14.4	429[r]	50.1
	22	4.02[a]	0.59	213[a]	27.0	262[b]	94.0
	23	1.80[b]	0.59	101[b]	27.0	191[b]	94.0
	24	2.25[b]	0.40	81.7[b]	18.1	339[b]	63.1
	25	1.80[b]	0.40	99.1[b]	18.1	671[a]	63.1
	26	1.75[b]	0.51	90.6[b]	23.4	274[b]	81.5
Low MEI	22	0.93[i]	0.81	90.6[i]	37.0	126	129.1
	23	1.04[i]	0.81	59.7[i]	37.0	142	129.1
	24	2.18[i]	0.56	84.9[i]	25.5	294	88.8
	25	2.01[i]	0.56	116[i]	25.5	610	88.8
	26	1.82[i]	0.56	95.1[i]	25.5	157	88.8
High MEI	22	7.10[h]	0.81	336[h]	37.0	398	129.1
	23	2.55[i]	0.81	143[i]	37.0	240	129.1
	24	2.33[i]	0.56	78.4[i]	25.5	385	88.8
	25	1.60[i]	0.56	82.1[i]	25.5	731	88.8
	26	1.69[i]	0.84	86.1[i]	38.4	390	133.8
Egg* (covariate)		0.27		30.7		37.2	
Source of variation		P-value					
Egg		0.59		0.18		0.64	
Treatment		0.001		0.007		0.022	
Age		0.028		0.004		0.001	
Treatment x Age		0.002		0.005		0.89	

[r-s] means within column within treatment with no common superscript differ ($P < 0.05$)

[a-b] means within column within age with no common superscript differ ($P < 0.05$)

[h-i] means within column within treatment x age with no common superscript differ ($P < 0.05$)

*The covariate egg was 0 for birds that had not laid an egg and was 1 for birds that had laid an egg

Table 5-8. Proportional carcass composition of broiler breeder pullets on Low ME intake (Low MEI) and High ME intake (High MEI) feeding treatments from 22 to 26 wk of age

Treatment	Age (wk)	Protein	SEM	Lipid	SEM	Ash	SEM	Water	SEM
		% of live BW							
Low MEI		20.5	0.3	10.3^{s}	0.4	3.17	0.12	65.5	0.6
High MEI		19.8	0.3	13.9^{r}	0.4	3.24	0.13	64.0	0.6
	22	17.8^{c}	0.5	9.5^{b}	0.8	2.89	0.24	69.3^{a}	1.1
	23	20.1^{b}	0.5	12.4^{a}	0.8	3.06	0.24	65.1^{b}	1.1
	24	20.2^{b}	0.4	13.4^{a}	0.6	3.26	0.17	64.1^{bc}	0.8
	25	21.9^{a}	0.4	11.9^{a}	0.5	3.59	0.16	62.4^{c}	0.7
	26	20.8^{ab}	0.5	13.3^{a}	0.7	3.24	0.21	62.6^{bc}	1.0
Low MEI	22	17.5	0.7	9.3^{k}	1.1	2.61	0.34	69.7	1.5
	23	20.3	0.7	9.7^{k}	1.1	3.15	0.34	65.8	1.5
	24	21.0	0.6	10.3^{k}	0.8	3.49	0.25	65.2	1.1
	25	22.5	0.5	10.6^{k}	0.7	3.44	0.22	62.7	1.0
	26	21.1	0.5	11.7^{jk}	0.8	3.18	0.23	63.8	1.0
High MEI	22	18.2	0.7	9.8^{k}	1.1	3.17	0.34	68.9	1.5
	23	19.8	0.7	15.1^{hi}	1.1	2.97	0.34	64.5	1.5
	24	19.4	0.6	16.5^{h}	0.8	3.03	0.25	63.0	1.1
	25	21.4	0.5	13.2^{ij}	0.8	3.74	0.23	62.0	1.0
	26	20.5	0.8	14.8^{hi}	1.2	3.30	0.35	61.5	1.6
Egg* (covariate)		0.32		1.05		-0.20		-1.16	
Source of variation		P-value							
Egg		0.49		0.14		0.35		0.22	
Treatment		0.11		< 0.001		0.71		0.07	
Age		< 0.001		0.005		0.16		0.001	
Treatment x Age		0.46		0.033		0.38		0.93	

$^{r-s}$ means within column within treatment with no common superscript differ ($P < 0.05$)

$^{a-c}$ means within column within age with no common superscript differ ($P < 0.05$)

$^{h-k}$ means within column within treatment x age with no common superscript differ ($P < 0.05$)

*The covariate egg was 0 for birds that had not laid an egg and was 1 for birds that had laid an egg

6 Chapter 6: Synthesis

6.1 General Discussion

Many successful feeding programs have been conducted for broiler breeders (Zuidhof et al., 2015; de Beer and Coon, 2007), however, there is still concern regarding broiler breeder welfare as they are severely feed restricted and only have access to feed once in 24 h or once every 48 h. During the most severe feed restriction, between 8 to 16 wk of age, feed intake is between 25 to 33% of the intake of *ad libitum* fed pullets (de Jong, 2006). A precision feeding system was developed at the University of Alberta to provide the right amount of feed to each bird, increase body weight (**BW**) uniformity, and increase efficiency through increasing consistency in nutrient supply because it provides several small meals in a day for an individual bird. The PF system individually weighs broiler breeders and makes decisions in real time about whether or not to feed them after comparing their actual BW with their target BW. It was shown that precision feeding (**PF**) pullets had lower cumulative feed conversion ratio (**FCR**) than conventional (**CON**) skip-a-day pullets and were more efficient (Chapter 3). The energetic efficiency of the CON daily restricted feeding and the PF hens was estimated using residual feed intake (**RFI**), and residual heat production (**RHP**; Chapter 3). Cumulative FCR was calculated by dividing BW gain by feed intake from 10 to 23 wk of age. However, RFI is a more precise indicator of efficiency compared to FCR because it accounts for feed intake, BW gain, and total HP. In most cases, high-producing animals have higher feed intake which results in lower efficiency (higher value for RFI), thus unfairly penalizing highly productive animals for the extra feed

required for higher levels of production (Zuidhof, 2019). Thus we estimated RHP as another efficiency factor. The RHP presents heat loss in a way that is unbiased by ME intake. The disadvantage of RFI and RHP is that both are complicated to estimate compared to FCR. Thus, RFI and RHP are not being widely used by poultry producers. However, it is suggested that poultry scientists use RHP as an energetic efficiency factor to identify the most efficient feeding systems without confounding factors. It is also suggested to poultry breeders who identify desirable traits for the next generations, to use RHP as an energetic efficiency factor to identify the most efficient individuals with higher egg production and lower total HP. In Chapter 3 and 4, ME intake models were developed to derive the ME requirements for total HP, average daily gain (**ADG**) and egg mass of broiler breeders.

The total HP was estimated as 120 kcal/kg$^{0.68}$ from 10 to 23 wk of age (Chapter 3) and 142 kcal/kg$^{0.67}$ from 23 to 34 wk of age (Chapter 4) in broiler breeders respectively. Pinchasov and Galili (1990) reported total HP as 207 kcal/kg$^{0.67}$ from 10 to 20 wk of age. Sakomura et al. (2003) reported total HP as 174 kcal/kg$^{0.75}$ from 9 to 20 wk of age. Reyes et al. (2012) estimated total HP as 110 kcal/kg$^{0.75}$ in broiler breeder hens from 32 to 42 wk of age. Pishnamazi et al. (2015) reported 136 kcal/kg$^{0.84}$ as total HP in broiler breeder hens from 25 to 41 wk of age. Obviously, the values of the estimated total HP among the current study and the previous studies vary. To have an idea about the trend of changes in total HP at different ages in broiler breeders, I compared the total HP (kcal) among the current study (2019, Chapter 3), Sakomura et al. (2003), and Pinchasov and Galili (1990)

for broiler breeders from 10 to 20 wk of age. The ME intake models were predicted as:

$MEI = (120 + u) \times BW^{0.68} + 1.52 \times ADG$ (Current study, 2019)

$MEI = 174 \times BW^{0.75} - 1.88 \times BW^{0.75} \times T + 2.83\ ADG$ (Sakomura et al., 2003)

$MEI = 207 \times BW^{0.67} + 0.71 \times ADG$ (Pinchasov and Galili, 1990)

Where MEI was ME intake (kcal/d); average of total HP per metabolic BW from 10 to 20 wk of age were respectively 120 kcal/kg$^{0.68}$, 174 kcal/kg$^{0.75}$, 207 kcal/kg$^{0.67}$; BW was kg; ADG was kcal/g; temperature (T) was at 21°C. The total HP (kcal/d) for the current study (2019, Chapter 3), Sakomura et al. (2003), and Pinchasov and Galili (1990) were calculated by multiplying the total HP (kcal/metabolic BW) by metabolic BW. Since these authors reported the exact BW from 10 to 20 wk of age, the total HP (kcal/d) was estimated from 10 to 20 wk of age (Figure 6-1). The average BW from 10 to 20 wk of age for the pullets in the study by Sakomura et al. (2003), Pinchasov and Galili (1990), and the current study were respectively 1,463; 1,529; and 1,475 g and broiler breeders in those studies were reared on the floor. The results of this comparison predicted that the total HP has been reduced by 44.3% in broiler breeder pullets from 1990 to 2019. The reduction in total HP over 29 years could be due to reducing the feed allocation for the broiler breeder. The decrease in feed intake of broiler breeders result in reducing total HP, in part due to reduction in diet-induced thermogenesis. The gap between the growth potential of broilers and broiler breeder target BW is increasing and as a result, the degree of feed restriction in broiler breeders became more severe (Renema et al., 2007b; Zuidhof, 2018).

These ME intake models can help farmers to predict feed allocations for their flock if farmers ask the feed company to report them the result of the NIR evaluations for the energy content of the diet. However, this method will not be practical for farmers if they do not know the energy content of the diets. Although using these ME intake models for feed allocation decisions is complicated, they result in precise predictions. For example if birds in a flock are 30 wk old and the average BW of the flock is 3,435 g and the farmer is attempting to reach the birds to target BW of 3,465 g at 31 wk old, the feed allocation using the ME intake model in Chapter 4 can be predicted as follows:

Predicted Model in Chapter 4:

$MEI = 142 \times BW^{0.67} + 1.75 \times ADG + 0.75 \times EM + \varepsilon$

The average BW from the period of 30 to 31 wk of age:

(3,435 g + 3,465 g) / 2 = 3,450 g = 3.450 kg

The expected maintenance requirement:

$142\ kcal/kg^{0.67}/d \times (3.450\ kg)^{0.67} = 325.6\ kcal/d$

The expected growth requirement:

ADG needed to attain the target BW at wk 31 = (3,465 g – 3,435 g) / 7 = 4.3 g/d

The expected growth requirement = 1.75 kcal/g × 4.3 g/d = 7.53 kcal/d

The expected egg mass requirement:

Expected egg mass from 30 to 31 wk of age=

(60 g egg × 85% egg production) /100 = 51 g/d

The expected egg production requirement = 0.75 kcal/g × 51 g/d = 38.3 kcal/d

The predicted ME intake from 30 to 31 wk of age = expected ME intake for maintenance + expected ME intake for growth + expected ME intake for egg mass

The predicted ME intake from 30 to 31 wk of age = 325.6 kcal/d + 7.53 kcal/d + 38.3 kcal/d = 371 kcal

ME of the diet used by the farmer = 2800 kcal/kg

Predicted average daily feed intake for the broiler breeders to reach 3,465 g at 31 wk old = (371 kcal/kg / 2800 kcal/kg) × 1000 g/kg = 133 g

Thus, using the predicted model in Chapter 4, the expected feed allocation from 30 to 31 wk of age would be 133 g/d.

The estimated ME requirement for egg mass in the current study (0.75 kcal/g; Chapter 4) was lower compared to reported values by Reyes et al. (2012; 2.30 kcal/g) and Pishnamazi et al. (2015; 1.79 kcal/g). The empirical model did not predict a realistic ME cost for each g of egg mass (Chapter 4). The reasons could be due to the relatively small number of experimental units and the short duration of egg collection. Thus, a larger sample size and a longer duration of egg production (for example from 23 to 55 wk of age) to predict a precise value for ME_e. It is possible that the ME intake model overestimated the ME requirement for total HP and underestimated the ME_g and ME_e.

In broiler breeders, body composition has changed as a consequence of selection programs (Figure 6-2). Bennett and Leeson (1990) reported a 22-wk total carcass fat level of 13.9% using a standard diet in broiler breeders. Miles et al. (1997) reported 22-wk total carcass lipid level at 11.6% using a standard diet in

broiler breeders. Renema et al. (2007a) reported a 22-wk total carcass fat level of 13.4% using a standard diet in broiler breeders. van Emous et al. (2015) reported 22-wk total carcass lipid level at 7.3% using a standard diet in broiler breeders. Zuidhof (2018) predicted total carcass lipid level at 6.9% using a standard diet in broiler breeders. In Chapter 4, it was estimated that carcass lipid level was at 5% using a standard diet in broiler breeders from 23 to 34 wk of age. Looking back through the literature over time, it is clear that carcass fat in broiler breeders has been decreasing. A 64% decrease in carcass fat levels observed in the current experiment (2019) compared with Bennett and Leeson (1990), due to severe feed restriction raises concerns that leanness may negatively affect reproductive success in broiler breeders.

In broiler breeders, onset of sexual maturation under adequate photostimulation can be affected by different factors such as age (hypothalamic-pituitary gonadal (**HPG**) axis maturation), BW, carcass composition, and concentration of reproductive hormones in broiler breeders (Renema et al., 1999a,b). However, there is lack of information about the effect of ME intake on the molecular mechanisms that affect onset of sexual maturation in broiler breeders. Our study provided new information for poultry scientists that under adequate photostimulation, higher ME intake increased the expression of gonadotropin releasing hormone (**GnRH-I**), GnRH receptor I (**GnRH-RI**), and luteinizing hormone (**LH**) genes and accelerated sexual maturation in broiler breeder pullets. This information might be useful for reproductive scientists in other species as well. Moreover, High ME intake increased the plasma concentration of reproductive

hormones (LH, follicle stimulating hormone (**FSH**), and estradiol (**E2**)) in breeders and increased carcass lipid in pullets at the onset of lay (Chapter 5). However, research needs to be carried out to evaluate the effect of increasing BW and increasing the ratio of energy to protein on stimulating sexual maturation and maximum egg production in broiler breeders. Broiler breeders fed low ME diet (2,600 kcal/kg) showed a delayed age of sexual maturity of 1.2 and 1.6 d compared to high ME (3,000 kcal/kg) and standard ME (2,800 kcal/kg) diets, respectively (van Emous et al., 2015). It was reported that broiler breeders fed low protein diets (13.81% from 15 to 42 d; 11.32% from 43 to 105 d; 12.62% from 106 to 154 d) had better persistency in lay than high protein diets (16.65% from 15 to 42 d; 14.15% from 43 to 105 d; 15.11% from 106 to 154 d), which might be explained by a higher proportion of abdominal fat and lower proportion of breast muscle at the end of rearing (van Emous et al., 2015). Breeders with a higher body fat content are probably more able to mobilize energy reserves in periods of a negative energy balance (Renema et al., 2013). The ratio of energy to protein is an important factor in broiler breeder feed formulation. To optimize dietary protein and energy levels, this subject requires extensive and systematic research.

The effect of increasing BW on stimulating sexual maturation in broiler breeders was evaluated by van der Klein et al. (2018) using 2 treatments: 1) pullets being reared following the standard breeder-recommended target BW curve (Aviagen, 2016), or a high target BW curve reaching the 21 wk BW at 18 wk (22% heavier than the Standard target BW at 21 wk of age). It was concluded that the High BW treatment advanced the onset of sexual maturation by 16.6 d compared

to the Standard BW treatment (van der Klein et al., 2018). It was suggested that target BW for breeder was too low for optimal sexual maturation after photostimulation. In Chapter 5, the ratio of the energy to protein was increased by 12.2%, additionally since the pullets in the High MEI treatment were fed unrestricted, they had 47% higher ME intake than pullets in the Low MEI. As a result, the onset of sexual maturation advanced for the High MEI pullets. However, it is suggested to carry out new research to discover the optimal combination of target BW and ratio of energy to protein for reproductive efficiency in broiler breeders. For such a study, a 6 × 6 factorial arrangement of treatments is suggested, with pullets being reared following the breeder recommended target BW curve (Standard), or increasing target BW by 5, 10, 15, 20, 25, and 30%, and an increasing the ratio of energy to protein by 2, 4, 6, 8, 10, and 15%.

According to the results of Chapter 5 and one of the experiment in our group (van der Klein et al., 2018), the message to the industry and poultry scientists would be to relax feed restriction for PF broiler breeders and for conventionally fed broiler breeders to help them to increase their ME intake and body lipid deposition to increase their productivity. However, research is required to assess the optimal level of relaxing feed restriction for the optimal sexual maturation and the maximum egg production.

6.2 Novelty of Research

The precision feeding system was developed at the University of Alberta to provide the right amount of feed to each bird, increase BW uniformity, and increase efficiency due to more frequent availability of dietary nutrients because it provides

several small meals in a day for an individual bird. The PF system individually weighs free run broiler breeders and makes decisions in real time about whether or not to feed them after comparing their actual BW with their target BW. So far, different feeding programs have been used in broiler breeders to control growth (Zuidhof et al., 2015; de Beer and Coon, 2007). However, the effect of energy intake on hypothalamic maturation and carcass composition of broiler breeders fed using PF system was not assessed when we started our second experiment. This thesis was the first investigation into the physiological implications of broiler breeder precision feeding. In Chapter 5, for the first time the effect of ME intake was evaluated on the expression of POMC, NPY, and LEPR genes in broiler breeder pullets to understand energy balance regulated by the hypothalamus at the onset of sexual maturation (Chapter 5). The result of the experiment showed a positive correlation of POMC with GnRH-I and GnIH, and a negative correlation of POMC with LH (Chapter 5). As a result, it was hypothesized that POMC may play a role in linking the control of reproduction with the control of energy status in broiler breeders (Figure 6-3).

6.3 Study Limitations

The precision feeding station in the first experiment was an early prototype and we realized that one of the features could be improved. For instance, sometimes, the PF scales were broken down because the load cell used on the scales were quite sensitive and caused the scales to record wrong BW and feed intake values and we were required to replace the scale. Birds were weighed manually once per week throughout the trial to check consistency of the PF scale with another

source. To replace the scale, the station needed to be carried out of the pen and again carried into the pen after scale replacement and this might have increased stress levels in birds. Elevated levels of plasma corticosterone (as a sign of stress) in female birds decreased reproductive hormone concentrations and caused hormonal imbalance (Henriksen et al., 2011). Chronic stress and hormone imbalance in the body can negatively affect folliculogenesis and egg production. Thus, we learned from the first prototype of the PF stations used in the current thesis to improve some features in the second prototype of the PF stations such as decreasing the sensitivity of the load cells on the scales with improving the software used in the PF stations. However, Zuidhof (2018) reported that PF hens fed by the second prototype of the PF stations still had lower egg production than conventionally daily fed hens. It was hypothesized that metabolic changes in PF birds provided an insufficient metabolic trigger for sexual maturation (Zuidhof, 2018).

From 0 to 9 wk of age, broiler breeders in skip-a-day treatment and PF treatment were fed using pan feeders. However, at 10 wk of the age, the experiment initiated and birds in PF groups started to use PF system and skip-a-day birds were still fed on pan feeders. The changes in the feeding method of the PF birds from the conventional feeding to the PF system may have influenced the results of the study. Because the metabolism of the PF birds has been changed from a feeding program with a less frequent availability of dietary nutrients (daily restricted feeding program) to a feeding program with more frequent availability of dietary nutrients (PF system feeding program). In the conventional feeding from 0 to 9 wk of age,

PF pullets were fed each morning and feed allocation decisions were made based on the average BW of pullets in the group (pen) to maintain breeder recommended BW targets. However, from 10 to 34 wk of age, the PF birds started to use the PF system and each individual bird received several small meals throughout the day based on her individual BW.

Feed allocation decisions for broiler breeders are difficult and this challenge for the research team underscores this difficulty. The BW of the CON hens at the start of the laying period was lower than the PF hens (2,477 vs. 2,602 g respectively). Thus, the feed allocation for the CON hens was increased compared to PF hens, which resulted in their higher ME intake. This explains at least partially the differences in productivity of the CON and the PF birds during laying period.

For the second experiment, two treatments were: 1) Low energy diet fed restricted according to the breeder-recommended Ross 308 BW target (Aviagen, 2011) using a typical commercial diet (2,807 kcal/kg, Low MEI) 2) High energy diet fed unrestricted (3,109 kcal/kg, High MEI). The design of this experiment did not allow us to evaluate the effect of ME energy levels and BW separately. For future research it is suggested to evaluate the effect of dietary energy levels and BW separately to have a better understanding of the effect of ME intake on the onset of sexual maturation in broiler breeder pullets. Thus, for the future research it is suggested to have 6 treatments with different target BW (for example standard target BW, and increasing target BW for 5, 10, 15, 20, and 25%) but the same ratio of energy to protein and 6 treatments with different ratios of the energy to protein (for example standard diet, increasing the ratio of energy to protein for 2, 4, 6, 8,

and 10%) but the same target BW. The results would suggest an optimal combination of both BW and ME:CP ratios for optimal sexual maturation in broiler breeders.

In Chapter 5, to assess the effect of ME intake on the plasma concentration of reproductive hormones, blood samples were collected weekly from all birds. However, if it was also collected right before culling each individual bird who came to lay, it could provide the information about the concentration of reproductive hormones approximately at the point of lay.

6.4 Future Research

The results of the first study taken together with other studies conducted in our laboratory lead us to believe that the PF pullets had an insufficient metabolic trigger such as body fat deposition for sexual maturation. Thus, it is suggested to assess relaxing feed restriction by increasing target BW for PF birds to help them to increase fat deposition and egg production. Research is required to assess the level of relaxing feed restriction for the optimal sexual maturation and maximum egg and chick production in PF hens.

In broiler breeder pullets, the effect of ME intake was assessed on 3 neuropeptides that have roles in energy balance and 7 neuropeptides that have roles in reproduction (Chapter 5). However, there are other neuropeptides that also play roles in energy balance and reproduction and there is lack of information about their molecular mechanisms in birds especially under feed restriction program. Some of these neuropeptides are as follows:

6.4.1 Genes Involved in the Regulation of Energy Balance

6.4.1.1 Agouti-related Peptide

Agouti-related peptide (**AGRP**) is co-expressed with NPY in the arcuate nucleus of the hypothalamus and together they regulate feed intake and energy balance. Stimulation of NPY/AGRP-expressing (anabolic) neurons mediates a net increase in feed intake and energy storage (Richards, et al., 2010). In our study the NPY gene expression did not differ between the High MEI and the Low MEI treatments. However, since NPY and AGRP are co-expressed in the hypothalamus, I hypothesize that increasing ME intake would decrease the mRNA levels of AGRP to decrease ME intake in responses to energy balance.

6.4.1.2 Liver Kinase B1, Mouse Protein MO25, and STE20-related Adaptor Protein

Hypothalamic neurons, particularly NPY and POMC neurons are likely important components of the AMPK pathway and transmitting metabolic information to the control of GnRH secretion (Amstalden et al., 2011). The mechanism for down-regulation of POMC expression by Low MEI was not assessed (Chapter 5). It was hypothesized that down-regulation of POMC expression in Low MEI pullets occurred via AMPK activation. To understand energy homeostasis better in broiler breeders, it is suggested to evaluate the effect of ME intake on AMPK pathway activation. It was reported that AMPK is expressed in liver, brain, kidney, spleen, pancreas, duodenum, abdominal fat and hypothalamus of broiler chickens (Proszkowiec-Weglarz et al., 2006b). The AMPK pathway was evaluated by the gene expressions of liver kinase B1 (**LKB1**), mouse

protein MO25 **(MO25**), and STE20-related adaptor protein (**STRAD**) in broiler chickens. These 3 genes are the key genes for the AMPK pathway. I hypothesize that increasing ME intake would activate the AMPK pathway in the hypothalamus of broiler breeders to increase the expression of anorexigenic neuropeptides (such as POMC) and decrease the expression of orexigenic neuropeptides (such as NPY, AGRP) in response to energy balance. Because the hypothalamus is the key component of energy balance. Once the AMPK pathway is activated, it would increase or decrease the related neuropeptides to maintain the energy balance.

6.4.1.3 Leptin

Leptin stimulates the expression of POMC mRNA in mice (Schwartz et al., 1997), and in sheep (Backholer et al., 2010). Leptin gene expression in chicken is not exclusively localized in adipose tissue but is also expressed in the liver (Taouis et al., 1998). Leptin treatment decreased expression of NPY and AGRP in the arcuate nucleus of adult rats (Ahima et al., 1999). However, there is lack of information about the effect of leptin gene on POMC, NPY and AGRP in poultry. I hypothesize that increasing ME intake would increase leptin mRNA levels to decrease ME intake in response to energy balance. Moreover, I hypothesize that increasing leptin mRNA levels would be associated with increased POMC mRNA levels and decrease NPY mRNA levels in response to energy balance. Assessing the effect of ME intake on leptin, LEPR, NPY, AGRP, and POMC gene expressions at the same time will help poultry scientists to understand the linkage among these genes and their functions in energy balance and their possible function in sexual maturation.

6.4.1.4 Melanocyte Stimulating Hormone Alpha and Melanocortin Receptor Type 4

In mammals, kisspeptin neurons have a role as effector neurons integrating metabolic and gonadal steroid feedback effects on GnRH secretion at the time of puberty (Amstalden et al., 2011). However, it is well established that chickens lack the kisspeptin (Joseph et al., 2013; Pasquier et al., 2014). The melanocortin system is considered to have an important role in mediating the neuroendocrine control of metabolism and reproductive function (Schneider, 2004). Melanocyte stimulating hormone alpha (**α-MSH**) is one of the products of the proopiomelanocortin (POMC) gene in the hypothalamus and it suppresses feed intake in chickens (Saneyasu et al., 2011). I hypothesize that increasing ME intake would increase mRNA levels of α-MSH in broiler breeders to decrease ME intake in response to energy balance. The effects of melanocortins in stimulating hypothalamic pituitary function seem to be mediated primarily by the melanocortin receptor type 4 (**MCR-4)** in the hypothalamus (Watanobe et al., 1999).

6.4.1.5 Corticotropin Releasing Hormone

MCR-4, and α-MSH, produced by stimulated POMC-expressing neurons, would activate additional neural pathways mediated by corticotropin releasing hormone (**CRH**) neurons and thyrotropin releasing hormone (**TRH**) neurons in hypothalamus (Richards, 2003). Anterior pituitary releases adrenocorticotropic hormone (**ACTH**) in response to CRH, and cells in the adrenal cortex release the glucocorticoid hormone corticosterone in response to ACTH in birds (Ritchie and Pilny, 2008; Bureau et al., 2009). Short-term HPA activity is assessed by measuring

corticosterone in blood and shows that HPA axis is responsible for the neuroendocrine adaptation component of the stress response (Bureau et al., 2009). Corticosteroids (corticosterone and cortisol) increase availability of all fuel substrates by mobilization of glucose, free fatty acids, and amino acids from endogenous stores and increase energy expenditure (Brillon, et al., 1995). I hypothesize that in broiler breeders increasing ME intake would increase mRNA levels of ACTH to decrease ME intake in response to energy balance and stimulation of ACTH mRNA levels would increase corticosterone concentration in the blood.

6.4.2 Genes Involved in the Regulation of Energy Balance and Reproductive Axis

6.4.2.1 Thyroid-stimulating Hormone

Thyroid-stimulating hormone (**TSH**) in the pars tuberalis (PT) of anterior pituitary is regulated by TRH produced by the paraventricular nucleus of hypothalamus in chickens (Geris et al., 2002, 2003). The TSH stimulates the release of GnRH-I in the median eminence (Bédécarrats et al., 2016). I hypothesize that in broiler breeder pullets, POMC may directly increase the expression of GnRH or it may indirectly affect the expression of GnRH through TRH in the hypothalamus.

The CRH and TRH would work together to decrease feed intake, via the hypothalamus-pituitary-adrenal axis (**HPA axis**) or the hypothalamus-pituitary-thyroid axis (**HPT axis**; McMinn et al., 2000; Blevins et al., 2002; Woods et al., 1998). I hypothesize that increasing ME intake would increase mRNA levels of CRH and TRH to reduce ME intake in response to energy balance. Evaluating the

effect of ME intake on the expression of α-MSH, MCR-4, CRH, TRH, ACTH, TSH, GnRH genes at the same time will help poultry scientists to understand better how these genes work together and provides better understanding of energy balance.

6.4.2.2 Growth Hormone, and Growth Hormone Receptor

Growth hormone (**GH**) neuropeptide is expressed in the anterior pituitary of chickens and GH receptor (**GH-R**) neuropeptide is expressed in the ovary (Luna et al., 2014). Numerous investigators demonstrated that GH alters voluntary feed intake, but there are marked species differences. In pig, GH reduced feed intake (Klindt et al., 1998) alternatively, in rats GH increased feed intake (Azain et al., 1995). In broiler chickens, GH decreased voluntary feed intake (Wang et al., 2000). Moreover, GH controls reproductive tract development in chickens by stimulating progesterone production (Luna et al., 2014). The secretion of growth hormone (GH, somatotropin) from the somatotropes of the pituitary gland happens through hypothalamic–pituitary–somatotropic axis (**HPS axis**).

There is a lack of information about the effect of ME intake on GH and GH-R mRNA levels in broiler breeders and how their functions might change under the changes in ME intake. I hypothesize that increasing ME intake would increase the mRNA levels of GH and GH-R to decrease ME intake in response to energy balance. Moreover, I hypothesize that increasing ME intake would increase progesterone concentration in the blood and advance sexual maturation in broiler breeders. Thus, it would be interesting to evaluate the effect of ME intake on GH

and its receptor to understand their function in energy balance and reproduction under feed restriction in broiler breeders.

6.5 Overall Implications

To allocate feed to broiler breeders in the conventional method, broiler breeders are typically weighed weekly (once or twice) and feed allocation decisions are based on their BW and rate of gain. However, a precise feed allocation requires weighing birds frequently, which requires labor (Schneider et al., 2005) or automated weighing equipment such as the PF systems. The PF system is capable of weighing each individual bird and allocates feed for each bird in real time. The next step for PF system would be commercialization of such technology for hatching egg producers. The PF system for the industry would facilitate more intricate genetic selection procedures due to the possibility of identifying desirable traits or behaviors at the individual bird level.

As observed in Chapter 3 and Chapter 4, on average, the PF birds had lower ME intake than the CON birds. Feed increases for the CON birds resulted in receiving a relative excess of energy for some birds that allowed them to come into production and increased egg production. The CON hens compared to the PF hens had higher fat pad content at 23 and 28 wk of age, greater ovary and stroma weight at 28 and 32 wk of age, and greater oviduct weight at 28 wk of age. These results suggest that the CON hens had more resources available for egg production and partitioned more energy toward developing ovary development and egg production. Conversely, feed allocation decisions for the PF treatment were based on the individual BW of each hen, and production-related increases in feed allocation were

provided after hens laid an egg. Thus, the PF hens had limited nutrients to form their first egg. We hypothesized that if PF birds are managed only according to BW, their target BW should be increased before the laying period and around the time of sexual maturation to help the PF birds to increase their ME intake and the body fat deposition to increase their productivity, especially around the onset of lay.

It was shown in Chapter 5, the High MEI treatment (unrestricted feeding with high energy diet) increased body lipid deposition, increased the expression of GnRH and GnRH-RI genes, increased the concentration of reproductive hormones, advanced the HPG axis maturation and accelerated sexual maturation compared to Low MEI treatment. These results indicate that relaxing feed restriction and increased ME intake may increase metabolic triggers for the onset of sexual maturation and increase egg production in PF broiler breeders. However, research is required to find the optimal level of feed restriction to achieve reproductive success.

6.6 Conclusion

From 10 to 23 wk of age, the pullets fed using the CON-skip-a-day feeding had higher ME intake than the pullets fed using the PF system. The CON-skip-a-day feeding probably required extra energy for the pullets to store and mobilize nutrients, and to deposit fat in their body compared to the PF system. At 23 wk of age, the CON-skip-a-day feeding was switched to CON-daily restricted feeding. The CON-daily restricted hens had higher ME intake from 23 to 28 wk of age, higher fat pad content at 23 and 28 wk of age, greater ovary and stroma weight at 28 and 32 wk of age, and greater oviduct weight at 28 wk of age. Feed increases for

the CON-daily restricted hens resulted in receiving an excess of energy for some birds that allowed them to come into the production and increased the egg production. In contrast, the feed allocation decisions for the PF treatment were based on the individual BW of each hen, and the increase in feed allocation was provided after the hen laid the egg; thus, the PF hens had limited nutrients to form eggs. The CON hens likely had more resources available for egg production and partitioned more energy toward developing reproductive tissues. Relaxing feed restriction increased ME intake, increased body lipid deposition, increased plasma concentration of reproductive hormones, advanced HPG axis maturation, and advanced the onset of sexual maturation in precision fed broiler breeder pullets. Therefore, relaxing feed restriction for modern broiler breeder hens would provide triggers for the onset of sexual maturation earlier and may increase egg production. However, research is required to assess the optimal level of relaxing feed restriction for optimal sexual maturation and maximum egg (and chick) production in broiler breeders.

6.7 References

Ahima, R. S., J. Kelly, J. K. Elmquist, and J. S. Flier. 1999. Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinemia. Endocrinol. 140:4923–4931.

Amstalden, M., B. R. C. Alves, S. Liu, R. C. Cardoso, and G. L. Williams. 2011. Neuroendocrine pathways mediating nutritional acceleration of puberty: insights from ruminant models. Front. Endocrinol. Lausanne. 2:1–7.

Azain, M. J., T. J. Roberts, R. J. Martin, and T. R. Kasser. 1995. Comparison of daily versus continuous administration of somatotropin on growth rate, feed intake, and body composition in intact female rats. J. Anim. Sci. 73:1019–1029.

Backholer, K., M. Bowden, K. Gamber, C. Bjørbæk, J. Iqbal, and I. J. Clarke. 2010. Melanocortins mimic the effects of leptin to restore reproductive function in lean hypogonadotropic ewes. Neuroendocrinology. 91:27–40.

Bédécarrats**,** G. Y, M. Baxter, and B. Sparling. 2016. An updated model to describe the neuroendocrine control of reproduction in chickens. Gen. Comp. Endocrinol. 227:58–63.

Blevins, J. E., M. W. Schwartz, and D. G. Baskin. 2002. Peptide signals regulating food intake and energy homeostasis. Can. J. Physiol. Pharmacol. 80:396–406.

Bennett, C. D., and S. Leeson. 1990. Body composition of the broiler breeder pullet. Poult. Sci. 69:715–720.

Brillon, D. J., B. Zheng, R. G. Campbell, and D. E. Matthews. 1995. Effect of cortisol on energy expenditure and amino acid metabolism in humans. Amer. J. Physiol. 268:E501–513.

Bureau, C., C. Hennequet-Antier, M. Couty, and D. Guémené. 2009. Gene array analysis of adrenal glands in broiler chickens following ACTH treatment. BMC. Genomics. 10:1–8.

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927–1939.

de Jong, I. C., B. Jones. 2006. Feed restriction and welfare in domestic birds. Pages 120-135 in Feeding in Domestic Vertebrates. V. Bels, ed. CABI, Wallingford, United Kingdom.

Geris, K. L., B. De Groef, E. R. Kühn, V. M. Darras. 2003. In vitro study of corticotropin-releasing hormone-induced thyrotropin secretion: ontogeny and inhibition by somatostatin. Gen. Comp. Endocrinol. 132:272–277.

Geris, K. L., B. De Groef, S. P. Rohrer, S. Geelissen, E. R. Kühn, V. M. Darras. 2002. Identification of somatostatin receptors controlling growth hormone and thyrotropin secretion in the chicken using receptor subtype-specific agonists. J. Endocrinol. 177:279–286.

Henriksen, R., T. G. Groothuis, and S. Rettenbacher. 2011. Elevated plasma corticosterone decreases yolk testosterone and progesterone in chickens: Linking maternal stress and hormone-mediated maternal effects. Plos. One. 1–8.

Joseph, N. T., J. A. Tello, G. Y. Bédécarrats, R. P. Millar. 2013. Reproductive neuropeptides: prevalence of GnRH and KNDy neural signaling components in a model avian, Gallus gallus. Gen. Comp. Endocrinol. 190:134–143.

Klindt J., J. T. Yen, F. C. Buonomo, A. J. Roberts, and T. Wise. 1998. Growth, body composition and endocrine responses to chronic administration of insulin-like growth factor I and porcine growth hormone in pigs. J. Anim. Sci. 76:2368–2381.

Luna, M., C. G. Martínez-Moreno, M. S. Ahumada-Solórzano, S. Harvey, M. Carranza, C. Arámburo. 2014. Extrapituitary growth hormone in the chicken reproductive system. Gen. Compar. Endocrinol. 203:60–68.

McMinn, J. E., D. G. Baskin, and M. W. Schwartz. 2000. Neuroendocrine mechanisms regulating food intake and body weight. Obesity Rev. 1:37–46.

Miles, R. D., H. R. Wilson, and R. H. Harms. 1997. Protein intake of broiler breeder replacements and its effect on body composition and subsequent performance. J. Appl. Anim. Res. 11:25–36.

Pasquier, J., A. G. Lafont, K. Rousseau, B. Querat, P. Chemineau, S. Dufour. 2014. Looking for the bird kiss: evolutionary scenario in sauropsids. BMC. Evol. Biol. 14:1–18.

Pinchasov, Y., and D. Galili. 1990. Research note: Energy requirement of feed-restricted broiler breeder pallets. Poult. Sci. 69:1792–1795.

Pishnamazi, A., R. A. Renema, D. C. Paul, I. I. Wenger, and M. J. Zuidhof. 2015. Effects of environmental temperature and dietary energy on energy partitioning coefficients of female broiler breeders1. J. Anim. Sci. 93:4734–4741.

Proszkowiec-Weglarz, M., M. P. Richards, R. Ramachandran, and J. P. McMurtry. 2006b. Characterization of the AMP-activated protein kinase pathway in chickens. Comp. Biochem. Physiol. B. 143:92–106.

Renema, R. A., T. G. V. Moraes, and M. J. Zuidhof. 2013. Effects of broiler breeder nutrition on chick quality and broiler growth. Pages 212–222 in Proc. Second Int. Poult. Meat Congress, Antalya, Turkey.

Renema, R. A., F. E. Robinson, M. Newcombe, and R. I. Mckay. 1999a. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 1. Growth and carcass characteristics. Poult. Sci. 78:619–628.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I. McKay. 1999b. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 2. Ovarian morphology and plasma hormone profiles. Poult. Sci. 78:629–639.

Renema, R. A., F. E. Robinson, and M. J. Zuidhof. 2007a. Reproductive efficiency and metabolism of female broiler breeders as affected by genotype, feed allocation, and age at photostimulation. 2. Sexual maturation. Poult. Sci. 86:2267–2277.

Renema, R. A., M. E. Rustad, and F. E. Robinson. 2007b. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. World's Poult. Sci. J. 63:457–472.

Reyes, M. E., C. Salas, and C. N. Coon. 2012. Metabolizable energy requirements for broiler breeder in different environmental temperatures. Int. J. Poult. Sci. 11:453–461.

Richards, M. P. 2003. Genetic regulation of feed intake and energy balance in poultry. Poult. Sci. 82:907–916.

Richards, M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMurtry. 2010. Feed intake regulation for the female broiler breeder: In theory and in practice. J. Appl. Poult. Res. 19:182–193.

Ritchie, M., A. A. Pilny. 2008. The anatomy and physiology of the avian endocrine system. Vet. Clin. Exot. Anim. 11:1–14.

Sakomura, N. K., R. Silva, H. P. Couto, C. Coon, and C. R. Pacheco. 2003. Modeling metabolizable energy utilization in broiler breeder pullets. Poult. Sci. 82:419–427.

Saneyasu, T., K. Honda, H. Kamisoyama, Y. Nakayama, K. Ikegami, S. Hasegawa. 2011. Alpha melanocyte stimulating hormone plays an important role in the regulation of food intake by the central melanocortin system in chicks. Peptides. 32:996–1000.

Schneider, J. E. 2004. Energy balance and reproduction. Physiol. Behav. 81:289–317.

Schneider, B., M. Zuidhof, F. Robinson, and R. Renema. 2005. Allocating feed to female broiler breeders: Technical Bulletin #2. Accessed Oct. 2018. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/pou9452.

Schwartz, M. W., R. J. Seeley, S. C. Woods, D. S. Weigle, L. A. Campfield, P. Burn, and D. G. Baskin. 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes. 46:2119–2123.

Taouis, M., J. W. Chen, C. Daviaud, J. Dupont, M. Derouet, J. Simon. 1998. Cloning the chicken leptin gene. Gene. 208:239–242.

van der Klein, S. A. S., G. Y. Bédécarrats, F. E. Robinson, and M. J. Zuidhof. 2018. Early photostimulation at the recommended body weight reduced broiler breeder performance. Poult. Sci. 97:3736–3745.

van Emous, R. A., R. P. Kwakkel, M. M. van Krimpen, and W. H. Hendriks. 2015. Effects of dietary protein levels during rearing and dietary energy levels during lay on body composition and reproduction in broiler breeder females. Poult. Sci. 94:1030–1042.

Wang, X., J. R. Day, Y. Zhou, J. L. Beard, and R. Vasilatos-Younken. 2000. Evidence of a role for neuropeptide Y and monoamines in mediating the appetite-suppressive effect of GH. Endocrinol. 166:621–630.

Watanobe, H., T. Suda, J. E. Wikberg, and H. B. Schiöth. 1999. Evidence that physiological levels of circulating leptin exert a stimulatory effect on luteinizing hormone and prolactin surges in rats. Biochem. Biophys. Res. Commun. 263:162–165.

Woods, S. C., R. J. Seeley, D. Porte Jr., M. W. Schwartz. 1998. Signals that regulate food intake and energy homeostasis. Science. 280:1378–1383.

Zuidhof, M. J. 2018. Lifetime productivity of conventionally and precision-fed broiler breeders. Poult. Sci. 97:3921–3937.

Zuidhof, M. J. 2019. Precision feeding: measuring and modeling feed efficiency. Proc. 68th Ann. National Breeders Roundtable, St. Louis.

Zuidhof, M. J., D. E. Holm, R. A. Renema, M. A. Jalal, and F. E. Robinson. 2015. Effects of broiler breeder management on pullet body weight and carcass uniformity. Poult. Sci. 94:1389–1397.

6.8 Figures

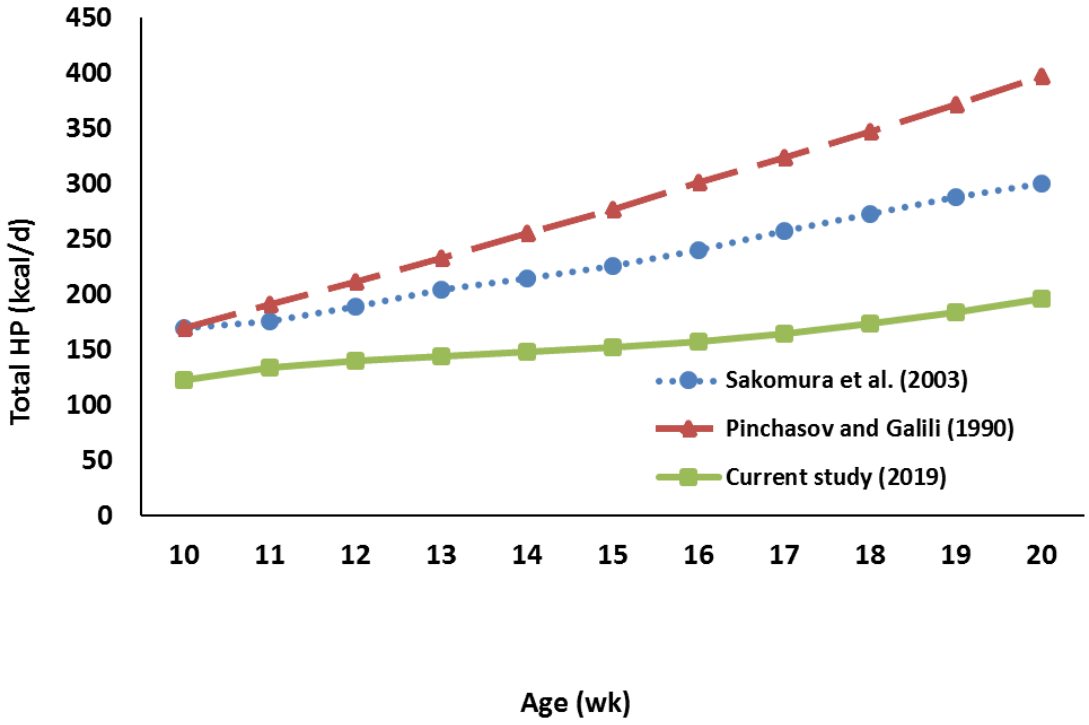

Figure 6-1. Comparison of estimated total heat production (HP, maintenance requirement) among the current study, Sakomura et al., (2003), and Pinchasov and Galili (1990) for broiler breeders. Total HP at each wk was estimated by the average total HP from 10 to 20 wk of age × metabolic BW using estimated ME intake (MEI) models: MEI = $BW^{0.75}$ × (186.52 - 1.94 × T), Hubbard Hi-Yield strain, from 9 to 14 wk of age, T was assumed 21°C; Sakomura et al., 2003; and MEI = $BW^{0.75}$ × (186.52 - 1.94 × T), Hubbard Hi-Yield strain, from 15 to 20 wk of age, T was assumed 21 °C, Sakomura et al., 2003; MEI = 207.5 × $BW^{0.67}$, Commercial Anak 2000 strain, Pinchasov and Galili, 1990; and MEI = 120 × $BW^{0.68}$, Ross 308 strain, Current study Chapter 3. BW was the average BW of birds and it was reported by authors for each wk separately from 10 to 20 wk of age.

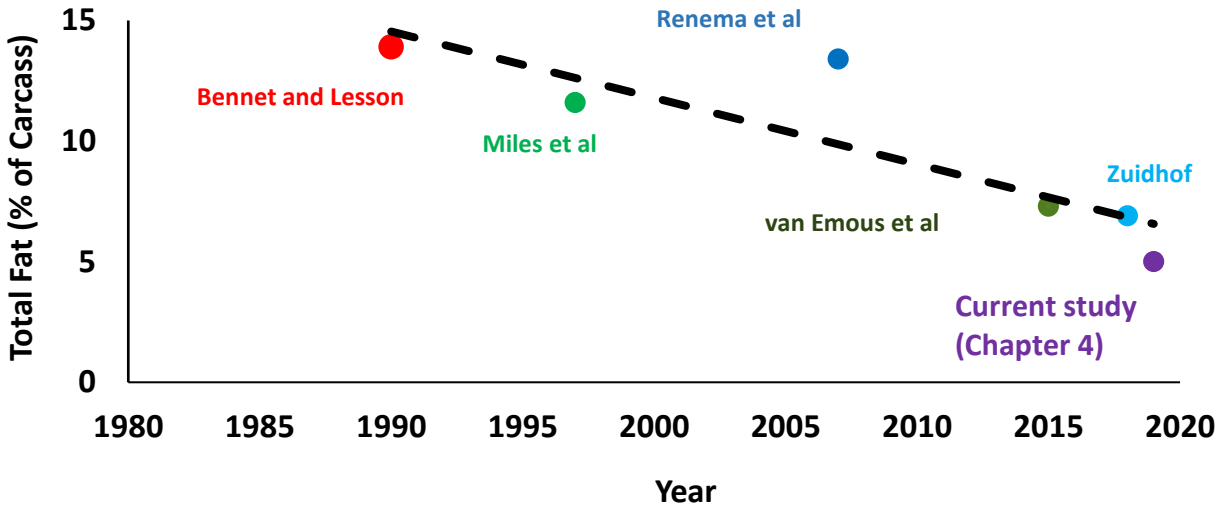

Figure 6-2. Comparison of body fat composition in broiler breeders among the current study (2019, Chapter 4), Bennett and Leeson (1990), Miles et al. (1997); Renema et al. (2007a); van Emous et al. (2015); Zuidhof (2018).

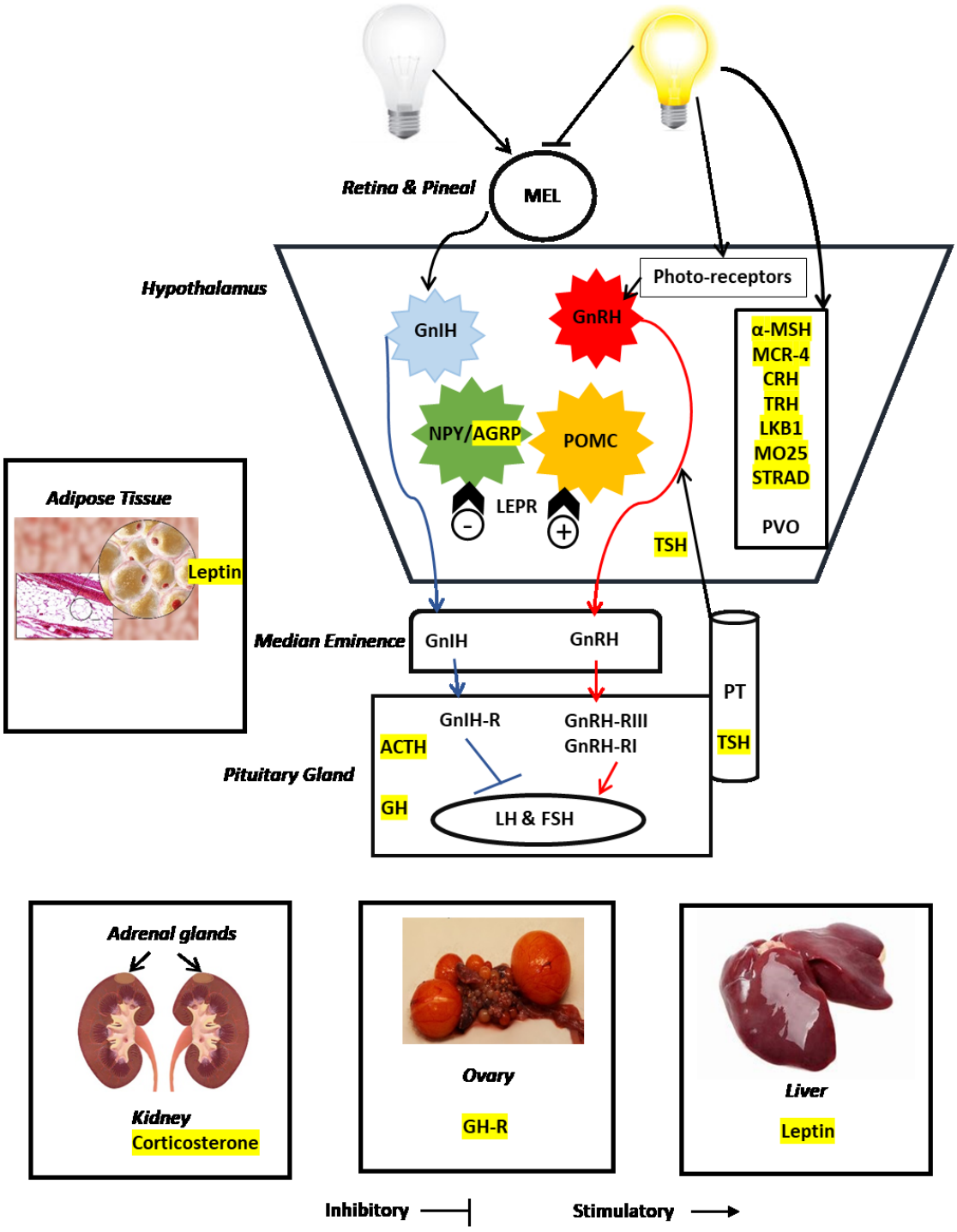
Retina & Pineal
MEL
Hypothalamus
Photo-receptors
GnIH
GnRH
α-MSH
MCR-4
CRH
TRH
LKB1
MO25
STRAD
PVO
NPY/AGRP
POMC
LEPR
TSH
Adipose Tissue
Leptin
Median Eminence
GnIH
GnRH
PT
TSH
GnIH-R
GnRH-RIII
GnRH-RI
ACTH
GH
Pituitary Gland
LH & FSH
Adrenal glands
Kidney
Corticosterone
Ovary
GH-R
Liver
Leptin
Inhibitory
Stimulatory

Figure 6-3. (Modified from Bédécarrats et al., 2016). The hypothalamic-pituitary-gonadal axis (HPG axis), the hypothalamus-pituitary-adrenal axis (HPA axis), the hypothalamic-pituitary-thyroid axis (HPT axis), and the hypothalamic-pituitary-somatotropic axis (HPS axis) are 4 axes in which the hypothalamus and pituitary direct neuroendocrine function. Gonadotropin inhibitory hormone (GnIH) and gonadotropin stimulatory hormone (GnRH) control the reproduction in birds. At the level of the hypothalamus, GnIH neurons directly inhibit GnRH-I synthesis and release. In addition, upon release into the median eminence, GnIH is delivered to the anterior pituitary gland via the portal vascular system where it inhibits the production of gonadotropins (Luteinizing hormone, LH and follicle stimulating hormone, FSH). During the scotophase (predominant under short days), melatonin (MEL) produced by the retina of the eye and the pineal gland stimulates the synthesis and release of GnIH resulting in the tonic inhibition of the reproductive axis. During the photophase (predominant under long days), a reduction in MEL lifts this inhibition while light directly stimulates hypothalamic photoreceptors. The HPG axis plays a critical part in the reproduction in birds. One group of photoreceptive cells located in the paraventricular organ (PVO) of the hypothalamus triggers the release of thyroid stimulating hormone (TSH) in the pars tuberalis (PT) of anterior pituitary gland which stimulates the release of GnRH in the median eminence. Moreover, TSH is regulated by thyrotropin-releasing hormone (TRH) produced by the hypothalamus. The HPT axis is responsible for the regulation of metabolism. To control energy homeostasis the hypothalamic melanocortin system is composed of two main populations of neurons, one set that expresses neuropeptide Y (NPY) and a second set that expresses proopiomelanocortin (POMC) that both have important roles in feed intake regulation. Increased expression of NPY stimulates feed intake and energy storage (anabolic), whereas increased expression of POMC reduces energy intake (catabolic). There are other genes that might have an important role in feed regulation and leptin is a prime candidate. Leptin gene is expressed in adipose tissue and in liver. Injection of leptin results in decreasing feed intake and decreasing BW. It is hypothesized that leptin binds to its receptor in the hypothalamus and when feed intake increases, leptin gene along to its receptor increase POMC and decreases NPY gene expressions to reduce feed intake in response to energy homeostasis. Hypothalamic neurons, particularly NPY and POMC neurons are important components of the AMP-activated protein kinase (AMPK) pathway. Key genes for the function of AMPK pathways are liver kinase B1(LKB1), mouse protein MO25 (MO25), and STE20-related adaptor protein (STRAD) and they are expressed in the hypothalamus. Melanocyte stimulating hormone alpha (α-MSH) is one of the products of the POMC gene in the hypothalamus and it suppresses feed intake in chickens. The effects of melanocortins in stimulating hypothalamic pituitary function seem to be mediated primarily by the melanocortin receptor type 4 (MCR-4). Working through MCR-4, α-MSH, produced by stimulated POMC-expressing neurons, would activate additional neural pathways mediated by corticotropin releasing hormone (CRH) neurons and TRH neurons in hypothalamus. Anterior pituitary releases adrenocorticotropic hormone (ACTH) in response to CRH, and cells in the adrenal cortex release the glucocorticoid hormone corticosterone in response to ACTH in birds. The HPA axis is responsible for the neuroendocrine adaptation component of the stress response. Agouti-related peptide (AGRP) is co-expressed with NPY in the arcuate nucleus of the hypothalamus and mediates a net increase in feed intake and energy storage. Growth hormone (GH) neuropeptide is expressed in anterior pituitary of chickens and GH receptor (GH-R) neuropeptide is expressed in ovary. In broiler chickens, GH decreases voluntary feed intake and the GH controls reproductive tract development in chickens by stimulating progesterone production. The secretion of growth hormone (GH, somatotropin) from the somatotropes of the pituitary gland happens through hypothalamic-pituitary-somatotropic axis (HPS axis).

References

Abeyrathne, E. D., H. Y. Lee, and D. U. Ahn. 2013. Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. Poult. Sci. 92:3292–3299.

Adachi, H., Y. Takemoto, T. Bungo, and T. Ohkubo. 2008. Chicken leptin receptor is functional in activating JAK-STAT pathway in vitro. J. Endocrinol. 197:335–342.

Aggrey, S. E., and R. Rekaya. 2013. Dissection of Koch's residual feed intake: implications for selection. Poult. Sci. 92:2600–2605.

Ahima, R. S., J. Kelly, J. K. Elmquist, and J. S. Flier. 1999. Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinemia. Endocrinol. 140:4923–4931.

Amstalden, M., B. R. C. Alves, S. Liu, R. C. Cardoso, and G. L. Williams. 2011. Neuroendocrine pathways mediating nutritional acceleration of puberty: insights from ruminant models. Front. Endocrinol. Lausanne. 2:1–7.

Andersen, C. L., J. L. Jensen, and T. F. Ørntoft. 2004. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer. Res. 64:5245–5250.

AOAC. 2007. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Arthur, J. P. F., and R. M. Herd. 2008. Residual feed intake in beef cattle. R. Bras. Zootec. 37:269–279.

Aviagen, 2018. Ross 308 parent stock management handbook. Aviagen Inc., Huntsville, AL. Accessed Feb 2019. http://en.aviagen.com/assets/Tech_Center/BB_Resources_Tools/Pocket_Guides/Ross-PS-Pocket-Guide2013EN.pdf.

Aviagen. 2011. Ross 308 parent stock: Performance objectives. Aviagen Inc., Huntsville, AL. Accessed Aug. 2018. http://en.aviagen.com/brands/ross/products/ross-308.

Aviagen. 2013. Ross 308 PS nutrition specifications. Aviagen Incorporated, USA. Accessed Nov. 2016. http://en.aviagen.com/brands/ross/products/ross-308.

Azain, M. J., T. J. Roberts, R. J. Martin, and T. R. Kasser. 1995. Comparison of daily versus continuous administration of somatotropin on growth rate, feed intake, and body composition in intact female rats. J. Anim. Sci. 73:1019–1029.

Backholer, K., M. Bowden, K. Gamber, C. Bjørbæk, J. Iqbal, and I. J. Clarke. 2010. Melanocortins mimic the effects of leptin to restore reproductive function in lean hypogonadotropic ewes. Neuroendocrinol. 91:27–40.

Bartov, I., S. Bornstein, Y. Lev, M. Pines, and J. Rosenberg. 1988. Feed restriction in broiler breeder pullets: skip-a-day versus skip two-days. Poult. Sci. 67:809–813.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83:189–204.

Baxter, M., N. Joseph, V. R. Osborne, and G. Y. Bedecarrats. 2014. Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. Poult. Sci. 93:1289–1297.

Becker, W. A., J. V. Spencer, L. W. Mirosh, and J. A. Verstrate. 1979. Prediction of fat and fat free live weight in broiler chickens using back skin fat, abdominal fat and live body weight. Poult. Sci. 58:835–842.

Bédécarrats, G. Y. 2015. Control of the reproductive axis: Balancing act between stimulatory and inhibitory inputs. Poult. Sci. 94:810–815.

Bédécarrats, G. Y., M. Baxter, and B. Sparling. 2016. An updated model to describe the neuroendocrine control of reproduction in chickens. Gen. Comp. Endocrinol. 227:58–63.

Bédécarrats, G.Y., M. Shimizu, and D. Guemene. 2006. Gonadotropin releasing hormones and their receptors in avian species. J. Poult. Sci. 43:199–214.

Bédécarrats**,** G. Y., H. McFarlane, S. R. Maddineni, and R. Ramachandran. 2009. Gonadotropin-inhibitory hormone receptor signaling and its impact on reproduction in chickens. Gen. Comp. Endocrinol. 163:7–11.

Bédécarrats**,** G. Y, M. Baxter, and B. Sparling. 2016. An updated model to describe the neuroendocrine control of reproduction in chickens. Gen. Comp. Endocrinol. 227:58–63.

Bédécarrats**,** G. Y. 2015. Control of the reproductive axis: Balancing act between stimulatory and inhibitory inputs. Poult. Sci. 94:810–815.

Bennett, C. D., and S. Leeson. 1990. Body composition of the broiler breeder pullet. Poult. Sci. 69:715–720.

Bentley, G. E., J. P. Jensen, G. J. Kaur, D. W. Wacker, K. Tsutsui, J. C. Wingfield. 2006. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). Horm. Behav. 49:550–555.

Bentley, G. E., N. Perfito, K. Ukena, K. Tsutsui, and J. C. Wingfield. 2003. Gonadotropin-inhibitory peptide in song sparrows (*Melospiza melodia*) in different reproductive conditions, and in house sparrows (*Passer domesticus*) relative to chicken-gonadotropin-releasing hormone. J. Neuroendocrinol. 15:794–802.

Bentley, G. E., T. Ubuka, N. L. McGuire, V. S. Chowdhury, Y. Morita, T. Yano, I. Hasunuma, M. Binns, J. C. Wingfield, K. Tsutsui. 2008. Gonadotropininhibitory hormone and its receptor in the avian reproductive system. Gen. Comp. Endocrinol. 156:34–43.

Blaxter, K. L. 1989. The utilization of the energy of food. Page 254–336 in Energy Metabolism in Animals and Man. Cambridge Univ. Press, Cambridge, England.

Blem, C. R. 1990. Current ornithology. Pages 59-113 in Avian Energy Storage. D. M. Power, ed. Plenum Press, New York, United States.

Blevins, J. E., M. W. Schwartz, and D. G. Baskin. 2002. Peptide signals regulating food intake and energy homeostasis. Can. J. Physiol. Pharmacol. 80:396–406.

Boswell, T., I. C. Dunn, and S. A. Corr. 1999. Hypothalamic neuropeptide Y mRNA is increased after feed restriction in growing broilers. Poult. Sci. 78:1203–1207.

Bowmaker, J. E., and R. M. Gous. 1989. Quantification of reproductive changes and nutrient requirements of broiler breeder pullets at sexual maturity. Br. Poult. Sci. 30:663–675.

Brillon, D. J., B. Zheng, R. G. Campbell, and D. E. Matthews. 1995. Effect of cortisol on energy expenditure and amino acid metabolism in humans. Amer. J. Physiol. 268:E501–513.

Bruggeman, V., O. Onagbesan, E. D'Hondt, N. Buys, M. Safi, D. Vanmontfort, L. Berghan, F. Vandesande, E. Decuypere. 1999. Effects of timing and duration of feed restriction during rearing on reproductive characteristics in broiler breeder females. Poult. Sci. 78:1424–1434.

Bureau, C., C. Hennequet-Antier, M. Couty, and D. Guémené. 2009. Gene array analysis of adrenal glands in broiler chickens following ACTH treatment. BMC. Genomics. 10:1–8.

Canadian Council on Animal Care. 2009. A guide to the care and the use of experimental animals. CCAC, Ottawa, ON, Canada.

Carling, D. 2004. The AMP-activated protein kinase cascade—A unifying system for energy control. Trends. Biochem. Sci. 29:18–24.

Chen, G., F. Yang, T. Wu, J. Jiang, and W. Zhou. 2016. The stimulatory effect of cerebral intraventricular injection of cNPY on precocial feeding behavior in neonatal chicks (*Gallus domesticus*). PLoS. One. 11:e0153342.

Cheung, C. C., J. E. Thornton, J. L. Kuijper, D. S. Weigle, D. K. Clifton, and R. A. Steiner. 1997. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinol. 138:855–858.

Chwalibog, A. 1991. Energetics of animal production. Acta Agric. Scand. 41:147–160.

Clarke, I. J., and J. T. Cummins. 1984. Direct pituitary effects of estrogen and progesterone on gonadotropin secretion in the ovariectomized ewe. Neuroendocrinol. 39:267–274.

Claus, R, and U Weiler. 1994. Endocrine regulation of growth and metabolism in the pig: a review. Livestock Prod. Sci. 37:245–260.

Close, W. H. 1990. The evaluation of feeds through calorimetry studies. Pages 21–39 in Feed stuff Evaluation. Butterworths. J. Wiseman D. J. A. Cole, ed. The University Press, Cambridge, United Kingdom.

Cobb-Vanress. 2005. Cobb 500 Breeder Management Guide: Blueprint for Success. Cobb-Vantress, Siloam Springs, AR.

Cobb Vantress. 2008. Cobb Broiler Management Guide: Blueprint for Success. Cobb-Vantress, Siloam Springs, AR.

Cocjin, B. B. 1990. Terms commonly used in feeding and nutrition. Pages 135–138 in Principles of Animal Science. Rex Book Store, Manila, Philippines.

Cota, D., K. Proulx, K. A. Smith, S. C. Kozma, G. Thomas, S. C. Woods, and R. J. Seeley. 2006. Hypothalamic mTOR signaling regulates food intake. Science. 312:927–930.

Couse, J. F., and K. S. Korach. 1999. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. 20:358–417.

Crews, D. H. D. 2005. Genetics of efficient feed utilization and national cattle evaluation: A review. Genet. Mol. Res. 4:152–165.

de Beer, M., and C. N. Coon. 2007. The effect of different feed restriction programs on reproductive performance, efficiency, frame size, and uniformity in broiler breeder hens. Poult. Sci. 86:1927–1939.

de Beer, M., and C. N. Coon. 2009. The effect of different feed restriction programs and dietary L-carnitine supplementation on reproductive performance, efficiency, frame size and uniformity in broiler breeder hens. Int. J. Poult. Sci. 8:409–425.

de Beer, M., R. W. Rosebrough, B. A. Russell, S. M. Poch, M. P. Richards, and C. N. Coon. 2007. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. Poult. Sci. 86:1726–1738.

de Beer, M., R. W. Rosebrough, B. A. Russell, S. M. Poch, M. P. Richards, and C. N. Coon. 2007. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder grower period. 1. Hepatic lipid metabolism. Poult. Sci. 86:1726–1738.

Decuypere, E., V. Bruggeman, N. Everaert, Y. Li, R. Boonen, J. de Tavernier, S. Janssens, and N. Buys. 2010. The broiler breeder paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. Br. Poult. Sci. 51:569–579.

de Jong, I. C., D. Guemene. 2011. Major welfare issues in broiler breeders. World. Poult. Sci. 67:73–81.

de Jong, I. C., B. Jones. 2006. Feed restriction and welfare in domestic birds. Pages 120-135 in Feeding in Domestic Vertebrates. V. Bels, ed. CABI, Wallingford, England.

Denbow, D. M. 1994. Peripheral regulation of food intake in poultry. J. Nutr. 124:1349S–1354S.

Denbow, D. M., S. Meade, A. Robertson, J. P. McMurtry, M. Richards, and C. Ashwell. 2000. Leptin-induced decrease in food intake in chickens. Physiol. Behav. 69:359–362.

Dunn, I. C., N. A. Ciccone, and N. T. Joseph. 2009. Endocrinology and genetics of the hypothalamic-pituitary-gonadal axis. Pages 61–88 in Biology of Breeding Poultry. P. M. Hocking, ed. CABI, Wallingford, Oxfordshire, England.

Eitan, Y., E. Lipkin, and M. Soller. 2014. Body composition and reproductive performance at entry into lay of anno 1980 versus anno 2000 broiler breeder females under fast and slow release from feed restriction. Poult. Sci. 93:1227–1235.

Elias, C. F., C. Asckenasi, C. Lee, J. Kelly, R. S. Ahima, C. Bjørbæk, J. K. Flier, C. B. Saper, and J. K. Elmquisk. 1999. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron. 23:775–786.

Emmans, G. C. 1994. Effective energy: a concept of energy utilization applied across species. Br. J. Nutr. 71:801–821.

Etches, R. J., and C. E. Duke. 1984. Progesterone, androstenedione and oestradiol content of the theca and granulosa tissues of the four largest ovarian follicles

during the ovulatory cycle of the hen (*Gallus domesticus*). Endocrinol. 103:71–76.

Fattori, T. R., H. R. Wilson, R. H. Harms, F. B. Mather, R. D. Miles, and G. D. Butcher. 1993. Response of broiler breeder females to feed restriction below recommended levels. 3. Characterizing the onset of sexual maturity. Poult. Sci. 72:2044–2051.

Friedman, J. M. 2011. Leptin and the regulation of body weight. Keio. J. Med. 60:1–9.

Friedman, J. M., and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature. 395:763–770.

Friedman-Einat M., L. A. Cogburn, S. Yosefi, G. Hen, D. Shinder, A. Shirak, and et al. 2014. Discovery and characterization of the first genuine avian leptin gene in the rock dove (*Columba livia*). Endocrinol. 155:3376–84.

Furuse, M. 2002. Central regulation of food intake in the neonatal chick. Anim. Sci. J. 73:83–94.

Furuse, M., H. Yamane, S. Tomonaga, Y. Tsuneyoshi, and D. M. Denbow. 2007. Neuropeptidergic regulation of food intake in the neonatal chick: A review. Jpn. Poult. Sci. 44:349–356.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, N. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. Br. Poult. Sci. 39:79–89.

Gabarrou, G. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. J. Nutr. 127:2371–2376.

Geraert, P. A., S. Guillaumin, A. Bordas and P. Merat. 1991. Evidence of a genetic control of diet-induced thermogenesis in poultry. Proc. 12th Symp. Eur. Anim. Prod. 58:380–383.

Geris, K. L., B. De Groef, E. R. Kühn, V. M. Darras. 2003. In vitro study of corticotropin-releasing hormone-induced thyrotropin secretion: ontogeny and inhibition by somatostatin. Gen. Comp. Endocrinol. 132:272–277.

Geris, K. L., B. De Groef, S. P. Rohrer, S. Geelissen, E. R. Kühn, V. M. Darras. 2002. Identification of somatostatin receptors controlling growth hormone and thyrotropin secretion in the chicken using receptor subtype-specific agonists. J. Endocrinol. 177:279–286.

Gilbert, A. B. 1971. The ovary. Pages 1163–1208 in The Physiology and Biochemistry of the Domestic Fowl. D. J. Bell and B. M. Freeman, ed. Academic Press, London, England.

Gilmet, T.E.R.M. 2015. Behavioural implications of precision feeding broiler breeders. MSc Diss. Univ. Alberta, Edmonton, Alberta.

Girard, T. E., M. J. Zuidhof, and C. J. Bench. 2017. Aggression and social rank fluctuations in precision-fed and skip-a-day-fed broiler breeder pullets. Appl. Anim. Behav. Sci. 187:38–44.

Goerzen, P. R., and W. L. Julsrud, and F. E. Robinson. 1996. Duration of fertility in ad libitum and feed-restricted caged broiler breeders. Poult. Sci. 75:962–965.

Gomes, R. C., R. D. Sainz, S. L. Silva, M. C. Ce´sar, M. N. Bonin, and P. R. Leme. 2012. Feedlot performance, feed efficiency reranking, carcass traits, body composition, energy requirements, meat quality and calpain system activity in Nellore steers with low and high residual feed intake. Livestock Sci. 150:265–273.

Guglielmo, C. G. 2018. Obese super athletes: fat-fueled migration in birds and bats. J. Exp. Biol. 221:pmid:29514885.

Hadinia, S. H., P. R. O. Carneiro, D. R. Korver, and M. J. Zuidhof. 2019. Energy partitioning by broiler breeder hens in conventional daily-restricted feeding and precision feeding systems. Poult. Sci. 0:1–12. http://dx.doi.org/10.3382/ps/pez387.

Hadinia, S. H., P. R. O. Carneiro, C. A. Ouellette, and M. J. Zuidhof. 2018. Metabolizable energy partitioning of broiler breeder pullets in skip-a-day and precision feeding systems. Poult. Sci. 97:4279–4289.

Hagan, M., P. A. Rushing, M. W. Schwartz, K. A. Yagaloff, P. Burn, S. C. Woods, R. J. Seeley. 1999. Role of the CNS melanocortin system in the response to overfeeding. J. Neurosci. 19:2362–2367.

Halaas, J. L., C. Boozer, J. Blair-West, N. Fidahusein, D. A. Denton, J. M. Friedman. 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. mice. Proc. Natl. Acad. Sci. USA. 94:8878–8883.

Heck, A., O. Onagbesan, K. Tona, S. Metayer, J. Putterflam, Y. Jego, J. J. Trevidy, E. Decuypere, J. Williams, M. Picard, and V. Bruggeman. 2004. Effects of ad

libitum feeding on performance of different strains of broiler breeders. Br. Poult. Sci. 45:695–703.

Hocking, P. M. 2006. High-fibre pelleted rations decrease water intake but do not improve physiological indexes of welfare in food-restricted female broiler breeders. Br. Poult. Sci. 47:19–23.

Hocking, P. M., R. Bernard, and G. W. Robertson. 2002. Effects of low dietary protein and different allocations of food during rearing and restricted feeding after peak rate of lay on egg production, fertility and hatchability in female broiler breeders. Br. Poult. Sci. 43:94–103.

Hocking, P. M., A. B. Gilbert, M. Walker, and D. Waddington. 1987. Ovarian follicular structure of white leghorns fed ad libitum and dwarf and normal broiler breeders fed ad libitum or restricted until point of lay. Br. Poult. Sci. 28:493–506.

Hocking, P. M., B. O. Hughes, and S. Keer-Keer. 1997. Comparison of food intake, rate of consumption, pecking activity and behaviour in layer and broiler breeder males. Br. Poult. Sci. 38:237–240.

Hocking, P. M., M. H. Maxwell, G. W. Robertson, and M. A. Mitchell. 2002. Welfare assessment of broiler breeders that are food restricted after peak of lay. Br. Poult. Sci. 43:5–15.

Horev, G., P. Einat, T. Aharoni, Y. Eshdat, and M. Friedman-Einat. 2000. Molecular cloning and properties of the chicken leptin-receptor (CLEPR) gene. Mol. Cell. Endocrinol. 162:95–106.

Hossain, M. A., A. F. Islam, P. A. Iji. 2012. Energy utilization and performance of broiler chickens raised on diets with vegetable proteins on conventional feeds. Asian J. Poult. Sci. 6:117–128.

Huang, J. X., L. Lu, L. Xi, X. G. Luo, and B. Liu. 2010. Effects of age and strain on the expression of leptin receptor, neuropeptide Y and pro-opiomelanocortin in the hypothalamus of young chickens. Br. Poult. Sci. 51:696–702.

Imai, K., and A. V. Nalbandov. 1978. Plasma and follicular steroid levels of laying hens after the administration of gonadotropins. Biol. Reprod. 19:779–784.

Jackson, S., and J. Diamond. 1996. Metabolic and digestive responses to artificial selection in chickens. Evolution 50:1638–1650.

Johnson, P. A. 2012. Follicle selection in the avian ovary. Reprod. Dom. Anim. 47:283–287.

Johnston, D. W. 1970. Caloric density of avian adipose tissue. Comp. Biochem. Physiol. 34: 827–832.

Jones, E. K. M., V. Zaczek, M. MacLeod, and P. M. Hocking. 2004. Genotype, dietary manipulation and food allocation affect indices of welfare in broiler breeders. Br. Poult. Sci. 45:725–37.

Joseph, N. T., K. Morgan, R. Sellar, D. McBride, R. P. Millar, and I. C. Dunn. 2009. The chicken type III GnRH receptor homologue is predominantly expressed in the pituitary, and exhibits similar ligand selectivity to the type I receptor. J. Endocrinol. 202:179–190.

Joseph, N. S., F. E. Robinson, R. A. Renema, and M. J. Zuidhof. 2002. Responses of two strains of female broiler breeders to a midcycle increase in photoperiod. Poult. Sci. 81:745–754.

Joseph, N. T., J. A. Tello, G. Y. Bédécarrats, R. P. Millar. 2013. Reproductive neuropeptides: prevalence of GnRH and KNDy neural signaling components in a model avian, Gallus gallus. Gen. Comp. Endocrinol. 190:134–143.

Katz, I. A., R. P. Millar, and J. A. King. 1990. Differential regional distribution and release of two forms of gonadotropin releasing hormone in the chicken brain. Peptides. 11:443–450.

Klindt J., J. T. Yen, F. C. Buonomo, A. J. Roberts, and T. Wise. 1998. Growth, body composition and endocrine responses to chronic administration of insulin-like growth factor I and porcine growth hormone in pigs. J. Anim. Sci. 76:2368–2381.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22:486–494.

Kriegsfeld, L. J., T. Ubukac, G. E. Bentley, and K. Tsutsui. 2015. Seasonal control of gonadotropin-inhibitory hormone (GnIH) in birds and mammals. Front. Neuroendocrinol. 37:65–75.

Kuenzel, W. J., L. W. Douglass, and B. A. Davison. 1987. Robust feeding following central administration of neuropeptide Y or peptide YY in chicks, Gallusdomesticus. Peptides. 8:823–828.

Kuenzel, W. J. 1994. Central neuroanatomical systems involved in the regulation of food intake in birds and mammals. J. Nutr. 124:1355S–1370S.

Kuenzel, W. J., M. M. Beck, and R. Teruyama. 1999. Neural sites and pathways regulating food intake in birds: A comparative analysis to mammalian systems. J. Exp. Zool. 283:348–364.

Leeson, S., and J. D. Summers. 1991. Feeding programs for broiler breeders. Pages 297–342 in Commercial Poultry Nutrition. 3rd ed. Nottingham. Univ. Press, Guelph, ON, Canada.

Leeson, S., and J. D. Summers. 2000. Nutrition and feeding. Pages 137–138 in Broiler Breeder Production. Nottingham Univ. Press, Thrumpton, Nottingham, England.

Leeson, S., and J. D. Summers. 2001. Energy. Pages 34–99 in Nutrition of the Chicken. 4th ed. Univ. Books, Guelph, ON, Canada.

Leffler, T. P., C. R. Moser, B. J. Mcmanus, and J. J. Urh. 2008. Determination of Moisture and Fat in Meats by Microwave and Nuclear Magnetic Resonance Analysis: Collaborative Study. J. AOAC. Int. 91:802–810.

Liu, X., I. C. Dunn, P. J. Sharp, and T. Boswell. 2007. Molecular cloning and tissue distribution of a short form chicken leptin receptor mRNA. Domest. Anim. Endocrinol. 32:155–166.

Liu, L., D. Li, E. R. Gilbert, Q. Xiao, X. Zhao, Y. Wang, H. Yin, Q. Zhu. 2015. Effect of monochromatic light on expression of estrogen receptor (ER) and progesterone receptor (PR) in ovarian follicles of chicken. PLoS ONE. 10:e0144102.

Liu, L., S. Xu, X. Wang, H. Jiao, J. Zhao, and H. Lin. 2016. Effect of dexamethasone on hypothalamic expression of appetite-related genes in

chickens under different diet and feeding conditions. J. Anim. Sci. Biotechnol. 7:23.

Long, Y. C., and J. R. Zierath. 2006. AMP-activated protein kinase signaling in metabolic regulation. J. Clin. Invest. 116:1776–1783.

Luiting, P., and E. M. Urff. 1991. Residual feed consumption in laying hens. 1. Quantification of phenotypic variation and repeatabilities. Poult. Sci. 70:1655–1662.

Luna, M., C. G. Martínez-Moreno, M. S. Ahumada-Solórzano, S. Harvey, M. Carranza, C. Arámburo. 2014. Extrapituitary growth hormone in the chicken reproductive system. Gen. Compar. Endocrinol. 203:60–68.

Maddineni, S., O. M. Ocon-Grove, S. M. Krzysik-Walker, G. L. Hendricks, J. A. Proudman, and R. Ramachandran. 2008. Gonadotrophin-inhibitory hormone receptor expression in the chicken pituitary gland: Potential influence of sexual maturation and ovarian steroids. J. Neuroendocrinol. 20:1078–1088.

McCue, M. D. 2006. Specific dynamic action: a century of investigation. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 144A:381–394.

McLean, B.K., N. Chang, and M.B. Nikitovitch-Winer. 1975. Ovarian steroids directly alter luteinizing hormone (LH) release by pituitary homografts. Endocrinol. 97:196–201.

McLeod, E. S., M. A. Jalal, and M. J. Zuidhof. 2014. Modeling ovarian follicle growth in commercial and heritage Single Comb White Leghorn hens. Poult. Sci. 93:2932–2940.

McMinn, J. E., D. G. Baskin, and M. W. Schwartz. 2000. Neuroendocrine mechanisms regulating food intake and body weight. Obesity Rev. 1:37–46.

Mench, J. A. 2002. Broiler breeders: Feed restriction and welfare. Worlds Poult. Sci. J. 58:23–29.

Merlet, F., J. Puterflam, J. M. Faure, P. M. Hocking, M. S. Magnusson, and M. Picard. 2005. Detection and comparison of time patterns of behaviours of two broiler breeder genotypes fed ad libitum and two levels of feed restriction. Appl. Anim. Behav. Sci. 94:255–271.

Microsoft, 2010. Accessed Mar. 2019. www.office.microsoft.com.

Miles, R. D., H. R. Wilson, and R. H. Harms. 1997. Protein intake of broiler breeder replacements and its effect on body composition and subsequent performance. J. Appl. Anim. Res. 11:25–36.

Miyamoto, K., Y. Hasegawa, M. Igarashi, N. Chino, S. Sakakibara, K. Kangawa, and H. Matsuo. 1983. Evidence that chicken hypothalamic luteinizing hormone-releasing hormone is [Gln2]-LH-RH. Life. Sci. 32:1341–1347.

Miyamoto, K., Y. Hasegawa, T. Minegishi, M. Nomura, Y. Takahashi, M. Igarashi, K. Kangawa, and H. Matsuo. 1982. Isolation and characterization of chicken hypothalamic luteinizing hormone-releasing hormone. Biochem. Biophys. Res. Commun. 107:820–827.

Mizuno, T. M., S. P. Kleopoulos, H. T. Bergen, J. L. Roberts, C. A. Priest, C. V. Mobbs. 1998. Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. Diabetes. 47:294–297.

Mohiti-Asli, M., M. Shivazad, M. Zaghari, M. Rezaian, S. Aminzadeh, and G. G. Mateos. 2012. Effects of feeding regimen, fiber inclusion, and crude protein content of the diet on performance and egg quality and hatchability of eggs of broiler breeder hens. Poult. Sci. 91:3097–3106.

Morrissey, K. L. H., T. Widowski, S. Leeson, V. Sandilands, A. Arnone, and S. Torrey. 2014. The effect of dietary alterations during rearing on growth, productivity, and behavior in broiler breeder females. Poult. Sci. 93:285–295.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens. Poult. Sci. 94:1611–1618.

Nangsuay, A., Y. Ruangpanit, R. Meijerhof, and S. Attamangkune. 2011. Yolk absorption and embryo development of small and large eggs originating from young and old breeder hens. Poult. Sci. 90:2648–2655.

National Research Council. 1981. Nutritional Energetics of Domestic Animals and Glossary of Energy Terms. 2nd ed. Natl. Acad. Press, Washington, DC, United States.

Nelson, D. L., and M. M. Cox. 2006. Bioenergetics and metabolism. Pages 481–488 in Lehninger Principle of Biochemistry. 4th ed. W. H. Freeman, Wisconsin, MDN, United States.

Noblet, J. 2013. Energy evaluation of pigs and poultry feeds: history, recent advances and applications to tropical conditions. 26th Annual PHILSAN Convention**,** Yapak, Boracay, Philippines.

Nonis, M. K., and R. M. Gous. 2016. Changes in the feather-free body of broiler breeder hens after sexual maturity. Anim. Product. Sci. 56:1099–1104.

Nonis, M. K., and R. M. Gous. 2012. Broiler breeders utilise body lipid as an energy source. S. Afr. J. Anim. Sci. 42:369–378.

NRC. 1981. Nutritional energetics of domestic animals and glossary of energy terms. Second Revised Edition, 1981 ed. National Academy Press, Washington, DC., United States.

Ohkubo, T., M. Tanaka, and K. Nakashima. 2000. Structure and tissue distribution of chicken leptin receptor (cOb-R) mRNA. Biochim. Biophys. Acta. 1491:303–308.

Onagbesan, O. M., S. Metayer, K. Tona, J. Williams, E. Decuypere, and V. Bruggeman. 2006. Effects of genotype and feed allowance on plasma luteinizing hormones, follicle-stimulating hormones, progesterone, estradiol levels, follicle differentiation, and egg production rates of broiler breeder hens. Poult. Sci. 85:1245–1258.

Paczoska-Eliasiewicz, H. E., A. Gertler, M. Proszkowiec, J. Proudman, A. Hrabia, A. Sechman, M. Mika, T. Jacek, S. Cassy, N. Raver, and J. Rzasa. 2003. Attenuation by leptin of the effects of fasting on ovarian function in hens (*Gallus demosticus*). Reproduction. 126:739–751.

Pasquier, J., A. G. Lafont, K. Rousseau, B. Querat, P. Chemineau, S. Dufour. 2014. Looking for the bird kiss: evolutionary scenario in sauropsids. BMC. Evol. Biol. 14:1–18.

Pearson, R. A., and K. M. Herron. 1981. Effects of energy and protein allowances during lay on the reproductive performance of broiler breeder hens. Br. Poult. Sci. 22:227–239.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucl. Acids Res. 29:e45.

Pinchasov, Y., and D. Galili. 1990. Research note: Energy requirement of feed restricted broiler breeder pallets. Poult. Sci. 69:1792–1795.

Pishnamazi, A., R. A. Renema, D. C. Paul, I. I. Wenger, and M. J. Zuidhof. 2015. Effects of environmental temperature and dietary energy on energy partitioning coefficients of female broiler breeders. J. Anim. Sci. 93:4734–4741.

Pishnamazi, A., R. A. Renema, M. J. Zuidhof, and F. Robinson. 2014. Effect of age at photostimulation on sexual maturation in broiler breeder pullets. Poult. Sci. 93:1274–1281.

Pishnamazi, A., R. A. Renema, M. J. Zuidhof, and F. E. Robinson. 2008. Effect of initial full feeding of broiler breeder pullets on carcass development and body weight variation. Poult. Sci. 17:505–514.

Pollock, D. L. 1999. A geneticist's perspective from within a broiler primary breeder company. Poult. Sci. 78:414–418.

Prokop, J. W., C. Schmidt, D. Gasper, R. J. Duff, A. Milsted, T. Ohkubo, and et al. 2014. Discovery of the elusive leptin in birds: identification of several “missing links” in the evolution of leptin and its receptor. PLoS. One. 9:e92751.

Proszkowiec-Weglarz, M., M. P. Richards, and J. P. McMurtry. 2006a. Molecular cloning, genomic organization and expression of three chicken 5′-AMP-activated protein kinase gamma subunit genes. Poult. Sci. 85:2031–2041.

Proszkowiec-Weglarz, M., M. P. Richards, R. Ramachandran, and J. P. McMurtry. 2006b. Characterization of the AMP-activated protein kinase pathway in chickens. Comp. Biochem. Physiol. B. 143:92–106.

Proszkowiec-Weglarz, M., and M. P. Richards. 2007. 5′-AMP-Activated protein kinase in avian biology. Avian. Poult. Biol. Rev. 18:123–145.

Pullar, J. D., and A. J. F. Webster. 1977. The energy cost of fat and protein deposition in the rat. Br. J. Nutr. 37:355–361.

Rabello, C. B. V., N. K. Sakomura, F. A. Longo, H. P. Couto, C. R. Pacheco, and J. B. K. Fernandes. 2006. Modelling energy utilisation in broiler breeder hens. Br. Poult. Sci. 47:622–631.

Radu-Rusu, R. M., M. G. Usturoi, C. G. Radu-Rusu, and I. VacaruOpris. 2012. Nutritional peculiarities of the hen eggs produced within improved cages or free range alternative systems. Lucrări Ştiinţifice-Seria Zootehnie. 57:201–205.

Reeds, P. J., K. W. J. Wahle, and P. Haggarty. 1982. Energy costs of protein and fatty acid synthesis. Proc. Nutr. Soc. 41:155–159.

Renema, R. A., and F. E. Robinson. 2004. Defining normal: Comparison of feed restriction and full feeding of female broiler breeders. Worlds Poult. Sci. J. 60:508–522.

Renema, R. A., F. E. Robinson, and P. R. Goerzen. 2001. Effects of altering growth curve and age at photostimulation in female broiler breeders. 1. Reproductive development. Can. J. Anim. Sci. 81:467-476.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I. McKay. 1999a. Effects of BW and feed allocation during sexual maturation in broiler breeder hens. 1. Growth and carcass characteristics. Poult. Sci. 78:629–639.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I. McKay. 1999b. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 2. Ovarian morphology and plasma hormone profiles. Poult. Sci. 78:629–639.

Renema, R. A., F. E. Robinson, and M. J. Zuidhof. 2007a. Reproductive efficiency and metabolism of female broiler breeders as affected by genotype, feed allocation, and age at photostimulation. 2. Sexual maturation. Poult. Sci. 86:2267–2277.

Renema, R. A., M. E. Rustad, and F. E. Robinson. 2007b. Implications of changes to commercial broiler and broiler breeder body weight targets over the past 30 years. World's Poult. Sci. J. 63:457–472.

Reyes, M. E., C. Salas, and C. N. Coon. 2011. Energy requirement for maintenance and egg production for broiler breeder hens. Int. J. Poult. Sci. 10:913–920.

Reyes, M. E., C. Salas, and C. N. Coon. 2012. Metabolizable energy requirements for broiler breeder in different environmental temperatures. Int. J. Poult. Sci. 11:453–461.

Richards, M. P. 2003. Genetic regulation of feed intake and energy balance. Poult. Sci. 82:907–916.

Richards, M. P., and M. Proszkowiec-Weglarz. 2007. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poult. Sci. 86:1478–1490.

Richards, M. P., R. W. Rosebrough, C. N. Coon, and J. P. McMurtry. 2010. Feed intake regulation for the female broiler breeder: In theory and in practice. J. Appl. Poult. Res. 19:182–193.

Ritchie, M., A. A. Pilny. 2008. The anatomy and physiology of the avian endocrine system. Vet. Clin. Exot. Anim. 11:1–14.

Robert, K. M., K. G. Daryl, A. M. Peter, and W. R. Victor. 2003. Pages in 12–21. Harper's Illustrated Biochemistry. 26th edn. McGraw-Hill companies Inc., London.

Robinson, F. E., and R. J. Etches. 1986. Ovarian steroidogenesis during follicular maturation in the domestic fowl (*Gallus domesticus*). Biol. Reprod. 35:1096–1105.

Robinson, F. E., G. M. Fasenko, R. A. Renema. 2003. Female reproduction: control of ovarian function. Pages 3-10 in Optimizing Chick Production in Broiler Breeders. F. E. Robinson, and R. A. Renema, ed. Spotted Cow Press, Edmonton, Canada.

Robinson, F. E., R. A. Renema, L. Bouvier, J. J. R. Feddes, J. L. Wilson, M. Newcombe, and R. I. McKay. 1998. Effects of photostimulatory lighting and

feed allocation in female broiler breeders. 1. Reproductive development. Can. J. Anim. Sci. 78:603–613.

Rodriguez, A. 2017. Effects of daytime and supplemental light spectrum on broiler breeder growth and sexual maturation. MSc thesis. University of Guelph, Guelph, Ontario.

Romero., L. F., J. S. Sands, S. E. Indrakumar, P. W. Plumstead, S. Dalsgaard, and V. Ravindran. 2014. Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease. Poult. Sci. 93:2501–2513.

Romero, L. F., M. J. Zuidhof, R. A. Renema, A. Naeima, and F. E. Robinson. 2009a. Characterization of energetic efficiency in adult broiler breeder hens. Poult. Sci. 88:227–235.

Romero, L. F., M. J. Zuidhof, R. A. Renema, A. Naeima, and F. E. Robinson. 2011. Effects of maternal energy efficiency on broiler chicken growth, feed conversion, residual feed intake, and residual maintenance metabolizable energy requirements. Poult. Sci. 90:2904–2912.

Romero, L. F., M. J. Zuidhof, R. A. Renema, F. E. Robinson, and A. Naeima. 2009b. Nonlinear mixed models to study metabolizable energy utilization in broiler breeder hens. Poult. Sci. 88:1310–1320.

Rubinstein, M., M. J. Low. 2017. Molecular and functional genetics of the proopiomelanocortin gene, food intake regulation and obesity. FEBS. Lett. 17:2593–2606.

Saiful, I. M., M. Fujita, and T. Ito. 2002. Effect of physical activities on heat production of white leghorn hens under different lighting regimes. J. Poult. Sci. 39:159–166.

Sakomura, N. K. 2004. Modeling energy utilization in broiler breeders, laying hens and broilers. Braz. J. Poult. Sci. 6:1–11.

Sakomura, N. K., R. Silva, H. P. Couto, C. Coon, and C. R. Pacheco. 2003. Modeling metabolizable energy utilization in broiler breeder pullets. Poult. Sci. 82:419–427.

Saneyasu, T., K. Honda, H. Kamisoyama, Y. Nakayama, K. Ikegami, S. Hasegawa. 2011. Alpha melanocyte stimulating hormone plays an important role in the regulation of food intake by the central melanocortin system in chicks. Peptides. 32:996–1000.

Savory, C. J., P. M. Hocking, J. S. Mann, and M. H. Maxwell. 1996. Is broiler breeder welfare improved by using qualitative rather than quantitative food restriction to limit growth rate? Anim. Welf. 5:105–127.

Savory, C. J., K. Marosz, and S. M. Rutter. 1993. Assessment of hunger in growing broiler breeders in relation to a commercial restricted feeding program. Anim. Welf. 2:131–152.

Schneider, J. E. 2004. Energy balance and reproduction. Physiol. Behav. 81:289–317.

Schneider, B., M. Zuidhof, F. Robinson, and R. Renema. 2005. Allocating feed to female broiler breeders: Technical Bulletin #2. Accessed Jul. 2019. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/pou9452.

Schwartz, M. W., R. J. Seeley, S. C. Woods, D. S. Weigle, L. A. Campfield, P. Burn, and D. G. Baskin. 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes. 46:2119–2123.

Scott, T. A., and F. Boldaji. 1997. Comparison of inert markers [chromic oxide or insoluble ash (Celite)] for determining apparent metabolizable energy of wheat- or barley-based broiler diets with or without enzymes. Poult. Sci. 76:594–598.

Seroussi, E., Y. Cinnamon, S. Yosefi, O. Genin, J. G. Smith, N. Rafati, and et al. 2016. Identification of the long-sought leptin in chicken and duck: expression pattern of the highly GC-rich avian leptin fits an autocrine/paracrine rather than endocrine function. Endocrinol. 157:737–751.

Sharma, V. K., S. S. Kundu, C. Datt, S. Prusty, M. Kumar, U. B. Sontakke. 2018. Buffalo heifers selected for lower residual feed intake have lower feed intake, better dietary nitrogen utilisation and reduced enteric methane production. J. Anim. Physiol. Anim. Nutr. 102:e607–e614.

Shaw, N. D., S. N. Histed, S. S. Srouji, J. Yang, H. Lee, and J. E. Hall. 2010. Estrogen Negative Feedback on Gonadotropin Secretion: Evidence for a Direct Pituitary Effect in Women. J. Clin. Endocrinol. Metab. 95:1955–1961.

Shils, M. E., M. Shike, A. C. Ross, B. Caballero, and R. J. Cousins. 2006. Energy needs: assessment and requirements. Pages 136–138 in Modern Nutrition in Health and Disease. N. F. Butte, and B. Caballero, ed. Lippincott Williams and Wilkins, New York, United States.

Shimizu, M., and G. Y. Bédécarrats. 2010. Activation of the chicken gonadotropin-inhibitory hormone receptor reduces gonadotropin releasing hormone receptor signaling. Gen. Comp. Endocrinol. 167:331–337.

Shimizu, M., and G. Y. Bédécarrats. 2006. Identification of a novel pituitary-specific chicken gonadotropin-releasing hormone receptor and its splice variants. Biol. Reprod. 75:800–808.

Silva, J. P., G. Lambert, D. van Booven, and C. Wahlestedt. 2016. Epigenomic and metabolic responses of hypothalamic POMC neurons to gestational nicotine exposure in adult offspring. Genom. Med. 8:93.

Summers, J. D., and S. Leeson. 1983. Factors influencing early egg size. Poult. Sci. 62:1155–1159.

Sun, Y. M., I. C. Dunn, E. Baines, R. T. Talbot, N. Illing, R. P. Millar, and P. J. Sharp. 2001. Distribution and regulation by oestrogen of fully processed and variant transcripts of gonadotropin releasing hormone I and gonadotropin releasing hormone receptor mRNAs in the male chicken. J. Neuroendocrinol. 13:37–49.

Swennen, Q., P.-J. Verhulst, A. Collin, A. Bordas, K. Verbeke, G. Vansant, E. Decuypere, and J. Buyse. 2007. Further investigations on the role of diet-induced thermogenesis in the regulation of feed intake in chickens: Comparison of age matched broiler versus layer cockerels. Poult. Sci. 86:895–903.

Taouis, M., J. W. Chen, C. Daviaud, J. Dupont, M. Derouet, J. Simon. 1998. Cloning the chicken leptin gene. Gene. 208:239–242.

Thomas, V. G., S. K. Mainguy, and J. P. Prevett. 1983. Predicting fat-content of geese from abdominal fat weight. J. Wildl. Manage. 47:1115–1119.

Tolkamp, B. J., and R. B. D'Eath. 2016. Hunger associated with restricted feeding systems. Pages 11-27 in Nutrition and the Welfare of Animals Farm. C. J. C. Phillips, ed. Springer, Cham, Switzerland.

Tolkamp, B. J., V. Sandilands, and I. Kyriazakis. 2005. Effects of qualitative feed restriction during rearing on the performance of broiler breeders during rearing and lay. Poult. Sci. 84:1286–1293.

Tsutsui, K. 2009. A new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH): Biosynthesis, mode of action and functional significance. Prog. Neurobiol. 88:76–88.

Tsutsui, K., G. E. Bentley, G. Bedecarrats, T. Osugi, T. Ubuka, and L. J. Kriegsfeld. 2010a. Gonadotropin-inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. Front. Neuroendocrinology. 31:284–295.

Tsutsui, K., G. E. Bentley, L. J. Kriegsfeld, T. Osugi, J. Y. Seong, H. Vaudry. 2010b. Discovery and evolutionary history of gonadotrophin-inhibitory hormone and kisspeptin: new key neuropeptides controlling reproduction. J. Neuroendocrinol. 22:716–727.

Tsutsui, K., E. Saigoh, K. Ukena, H. Teranishi, Y. Fujisawa, M. Kikuchi, S. Ishii, and P. J. Sharp. 2000. A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem. Biophys. Res. Commun. 275:661–667.

Tsutsui, K., T. Ubuka, G. E. Bentley, and L. J. Kriegsfeld. 2012. Gonadotropin-inhibitory hormone (GnIH): discovery, progress and prospect. Gen. Comp. Endocrinol. 177:305–314.

Ubuka, T., G. E. Bentley, K. Ukena, J. C. Wingfield, and K. Tsutsui. 2005. Melatonin induces the expression of gonadotropin-inhibitory hormone in the avian brain. Proc. Natl. Acad. Sci. U. S. A. 102:3052–3057.

van der Klein, S. A. S., G. Y. Bédécarrats, F. E. Robinson, and M. J. Zuidhof. 2018a. Early photostimulation at the recommended body weight reduced broiler breeder performance. Poult. Sci. 97:3736–3745.

van der Klein, S. A. S., G. Y. Bédécarrats, and M. J. Zuidhof. 2018b. The effect of rearing photoperiod on broiler breeder reproductive performance depended on body weight. Poult. Sci. 97:3286–3294.

van Emous, R. 2015. Body composition and reproduction in broiler breeders: impact of feeding strategies. PhD Diss. Univ. Wageningen, Wageningen, the Netherland.

van Emous, R. A., R. P. Kwakkel, M. M. van Krimpen, and W. H. Hendriks. 2015. Effects of dietary protein levels during rearing and dietary energy levels during lay on body composition and reproduction in broiler breeder females. Poult. Sci. 94:1030–1042.

Vanmontfort, D., L. R. Berghman, L. Rombauts, G. Verhoeven, and E. Decuypere. 1995. Developmental changes in immunoreactive inhibin and FSH in plasma of chickens from hatch to sexual maturity. Br. Poult. Sci. 36:779–790.

Vogtmann, H., P. Frirter, and A. L. Prabuck. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 16: 531–534.

Wang, X., J. R. Day, Y. Zhou, J. L. Beard, and R. Vasilatos-Younken. 2000. Evidence of a role for neuropeptide Y and monoamines in mediating the appetite-suppressive effect of GH. Endocrinol. 166:621–630.

Wang, T., C. C. Hung, and D. J. Randall. 2006. The comparative physiology of food deprivation: from feast to famine. Annu. Rev. Physiol. 2006. 68:223–251.

Wang, D., C. Xu, T. Wang, H. Li, Y. Li, J. Ren, and et al. 2016. Discovery and functional characterization of leptin and its receptors in Japanese quail (*Coturnix japonica*). Gen. Comp. Endocrinol. 225:1–12.

Watanobe, H., T. Suda, J. E. Wikberg, and H. B. Schiöth. 1999. Evidence that physiological levels of circulating leptin exert a stimulatory effect on luteinizing hormone and prolactin surges in rats. Biochem. Biophys. Res. Commun. 263:162–165.

Wei, Z., P. Li, S. Huang, P. Lkhagvagarav, M. Zhu, C. Liang, and C. Jia. 2019. Identification of key genes and molecular mechanisms associated with low egg production of broiler breeder hens in ad libitum. BMC. Genom. 20:408.

Welt, C. K, Y. L. Pagan, P. C. Smith, K. B. Rado, and J. E. Hall. 2003. Control of follicle-stimulating hormone by estradiol and the inhibins: critical role of estradiol at the hypothalamus during the luteal-follicular transition. J. Clin. Endocrinol. Metab. 88:1766–1771.

Wenk, C. 1997. Energy metabolism of farm animals. Pages 265–267 in Proceedings of the 14th Symposium on Energy Metabolism. K. J. McCracken, E. F. Unsworth, and A. R. G. Wylie. Newcastle, Co. Down, Northern Ireland. University Press, Cambridge.

Williams, J. 2012. Ovarian dysfunction in broiler breeder hens. Medycyna. Wet. 68: 131–138.

Wullschleger, S., R. Loewith, and M. N. Hall. 2006. TOR signaling in growth and metabolism. Cell 124:471–484.

Winder, W. W., and D. M. Thompson. 2007. Cellular energy sensing and signaling by AMP-activated protein kinase. Cell. Biochem. Biophys. 47:332–347.

Woods, S. C., R. J. Seeley, D. Porte Jr., M. W. Schwartz. 1998. Signals that regulate food intake and energy homeostasis. Science. 280:1378–1383.

Yang, S., Y. Suh, Y. M. Choi, S. Shin, J. Y. Han, K. Lee. 2013. Loss of fat with increased adipose triglyceride lipase-mediated lipolysis in adipose tissue during laying stages in quail. Lipids: 48:13–21.

Yang, S., Y. Suh, Y. M. Choi, S. Shin, J. Y. Han, K. Lee. 2013. Loss of fat with increased adipose triglyceride lipase-mediated lipolysis in adipose tissue during laying stages in quail. Lipids. 48:13–21.

Yang, N., C. Wu, I. McMillan. 1989. New mathematical model of poultry egg production. Poult. Sci. 68:476–481.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt. 1992a. Effect of feed allowance during rearing and breeding on female broiler breeders. 2. Ovarian morphology and production. Poult. Sci. 71:1750–1761.

Yu, M., F. E. Robinson, and A. R. Robblee. 1992b. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. Poult. Sci. 71:1739–1749.

Yuan, L., Y. Ni, S. Barth, Y. Wang, R. Grossmann, and R. Zhao. 2009. Layer and broiler chicks exhibit similar hypothalamic expression of orexigenic neuropeptides but distinct expression of genes related to energy homeostasis and obesity. Brain. Res. 1273:18–28.

Zuidhof, M. J. 2018. Lifetime productivity of conventionally and precision-fed broiler breeders. Poult. Sci. 97:3921–3937.

Zuidhof, M. J. 2019. A review of dietary metabolizable and net energy: Uncoupling heat production and retained energy. J. Appl. Poult. Res. 28:231–241.

Zuidhof, M. J., M. V. Fedorak, C. C. Kirchen, E. H. M. Lou, C. A. Ouellette, and I. I. Wenger. 2016. System and method for feeding animals. Precision ZX, Inc., assignee. US Pat. Application No.15/283,125.

Zuidhof, M. J., M. V. Fedorak, C. A. Ouellette, and I. I. Wenger. 2017. Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity. Poult. Sci. 96:2254–2263.

Zuidhof, M. J., D. E. Holm, R. A. Renema, M. A. Jalal, and F. E. Robinson. 2015. Effects of broiler breeder management on pullet body weight and carcass uniformity. Poult. Sci. 94:1389–1397.

Zuidhof, M. J., F. E. Robinson, J. J. R. Feddes, and R. T. Hardin. 1995. The effects of nutrient dilution on the well-being and performance of female broiler breeders. Poult. Sci. 74:441–456.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 20051. Poult. Sci. 93:2970–2982.

Appendix (Supplementary Materials)

Hormonal Analyses

Plasma LH and FSH Analyses

Approximately 2 mL of blood was taken by venipuncture of the brachial vein and collected in a sodium heparin blood vacutainer tubes (Evacuated glass tubes, Fisher Scientific, NH, USA). Blood plasma was recovered by centrifugation at 1244 × g for 15 min at 4°C and stored at −20°C until hormonal assay. Assay was performed in duplicates for every individual sample. Briefly, the micro ELISA plates were pre-coated with an antibody specific to LH. 100 µL of plasma and standards were added to the appropriate wells and incubated for 90 min at 37°C. Coating buffer was removed, wells were refilled with Biotinylated detection solution and assay plates were incubated for 1 h at 37°C. Plates were aspirated and washed with wash buffer (approximately 350 µL) 3 times. Horseradish peroxidase conjugate was then added to wells and incubated for 30 min at 37°C. Plates were aspirated and washed with wash buffer 5 times and incubated with substrate solution for 15 min at 37°C. After incubation, the reaction was terminated with 50 µL of stop solution (sulphuric acid) was added to terminate the reaction. The optical density was measured with a microplate spectrophotometer at 450 nm (Molecular Devices, California, USA). The standard curve and samples were plotted and analyzed using SoftMax® Pro (Version 5, Molecular Devices, USA). Plasma FSH concentration was quantified using the same general procedure as described above for LH except that the micro ELISA plates were pre-coated with an antibody specific to FSH.

Plasma E2 Analysis

To quantify E2 plasma concentration, prior to ELISA assay, E2 was extracted from plasma using ethanol and according to the method suggested by Baxter et al. (2014). Briefly, thawed samples were diluted with ethanol at 5:1 (ethanol: plasma) ratio. Samples were then vortexed, centrifuged for 5 min at 20°C at 1,800 × g, and frozen in a −80°C. The organic (ethanol) phase was recovered and transferred into new tubes, and dried using a SpeedVac (Thermo Savant SpeedVac SC210A Centrifugal Evaporator, Thermo Scientific, USA). Samples were reconstituted in half the original volume with assay buffer and stored at -20ºC until assay. Assay was performed in duplicates for every individual sample. Briefly, 50 µl of each extracted plasma sample was added in individual wells of microtiter ELISA plates coated with goat anti-rabbit IgG. Thereafter, 50 µl of horseradish peroxidase-labeled E2 and then 50 µl of detection antibody (anti-E2 antibody) was added to each well. Reagents were mixed gently and incubated for 1h at 37°C. Subsequently, wells were aspirated and washed 3 times with 350 µl of wash buffer. Next, 50 µl of substrate solution was added to each well and incubated for 30 min at 37°C. To terminate the reaction, 50 µl of stop solution (sulphuric acid) was added to each well. The optical density was measured with a microplate spectrophotometer at 450 nm (Molecular Devices, California, USA). The standard curve and samples were plotted and analyzed using SoftMax® Pro (Version 5, Molecular Devices, USA).