EFFECTS OF FEED RESTRICTION AND DURATION OF THE REPRODUCTION PERIOD ON REPRODUCTION HORMONES AND FOLLICULAR DEVELOPMENT IN BROILER BREEDER HENS

DISSE佘TATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

Han-Ken Liu, M.S.

The Ohio State University
2004

Dissertation Committee:
Professor Wayne L. Bacon, Adviser
Professor Karl E. Nestor
Professor Michael D. Lilburn
Professor Joseph S. Ottobre

Approved by

Adviser
Department of Animal Sciences
ABSTRACT

A strong negative association between growth rate (body weight) and female reproductive efficiency (egg production rate) in turkeys and chickens has been clearly illustrated. In commercial practice, body weight of broiler breeder hens is limited during the rearing and reproductive periods by restricting feed intake. This results in higher persistency and longer duration of settable egg laying in restricted-fed broiler breeder hens.

Production of settable eggs is increased by feed restriction, but declines as the duration of the reproductive period progresses in broiler breeder hens. The purpose of these studies was to examine the relationships of interval among preovulatory luteinizing hormone (LH) surges, characteristics of LH and progesterone (P₄) surges, estradiol-17β (E₂), leptin, and insulin concentrations during ovulatory cycles, duration of follicular development, and egg production rate in broiler breeder hens. Hens were subject to different feeding programs and sampled early (Early, 3 to 6 wk of production) and late (Late, after egg production had declined by about 25% at 19 to 31 wk of egg production) in the reproductive period.

In the first study (Chapter 2), a cannulation procedure to collect serial blood samples from broiler breeder hens was developed. Using this procedure for short (every 12 min for 36 h) and long term (hourly for 240 h) collections of blood samples, egg production rate of hens that continued to lay was not altered. In the second study
(Chapter 3), peripheral concentrations of LH, P₄, and E₂ was measured every 12 min for 36 h and no differences in hormonal surge characteristics or concentrations were found between feeding programs in ad libitum- and restricted-fed hens. In the third and fourth studies (Chapter 4 and 5, respectively), peripheral concentrations of LH, P₄, E₂, leptin, and insulin were measured hourly for 240 h early and late in the reproductive period in hens given different feeding programs. In the fifth study (Chapter 6), duration of follicular development was measured early and late in the reproductive period in hens given different feeding programs.

In the studies where the hens were bled hourly (Chapters 4, 5, and 6), one group of hens was ad libitum-fed during both growth and the reproductive periods (FF). A second group of hens was restricted-fed during the growth period and then switched to ad libitum feeding during the reproductive period (RF). A third group of hens was restricted-fed during both growth and the reproductive periods (RR). The program of feed restriction used for the second and third groups was based on recommendations of the supplier (Aviagen, 2000). The hens were cannulated and bled hourly to collect blood samples for 240 h early and late in the reproductive period. The Sudan IV (Red) and Black B were suspended in canola oil and one of the suspensions was forced-fed to hens daily to monitor duration of follicular development.

Total egg production rate (74 to 84% and 52 to 56%, respectively for Early and Late hens) was not different between feeding treatments within the Early and Late hens, but was higher in the Early hens. Production of abnormal eggs was not different between FF (0.92 egg 10 d⁻¹) and RF (0.50 egg 10 d⁻¹) hens, but was higher in the FF hens than in the RR hens (0.00 egg 10 d⁻¹). Feeding treatment had no effect on LH
surge interval within the Early and Late periods, but the interval was shorter in the Early (31.2 h) hens than in the Late (43.5 h) hens. Percentage blind LH surges (surges not associated with an oviposition) was not different among groups. The P₄ surge amplitude, overall E₂, leptin, and insulin concentrations were not different between Early and Late hens but P₄ baseline concentration was higher in the Early than Late hens. The P₄ baseline, P₄ surge amplitude, and overall E₂ and leptin concentrations were not different among feeding programs but insulin concentration was higher in RF and RR hens than in FF hens. A longer duration of ovarian follicular development in Late hens was associated with a lower total egg production rate but feed restriction had no effect on duration of ovarian follicular development in broiler breeder hens.

From these studies it was concluded that: 1) cannulation procedures and short term and long term serial bleeding were successfully developed for broiler breeder hens; 2) the concentrations or peripheral patterns of LH, P₄, and E₂ were not different during preovulatory surges between ad libitum and restricted-fed hens; 3) feeding treatment affected abnormal egg production, but had no effect on interval between LH surges; 4) mean LH surge interval was shorter in Early than Late hens; 5) leptin and insulin concentrations were not associated with differences in body weight, fat pad weight, interval between LH surges, and total egg production; and 6) a longer duration of follicular development in the Late hens was associated with a lower total egg production rate.
Dedicated to My Wife, LingBei, Daughter, Emily, and Parents
ACKNOWLEDGMENTS

The research presented in this dissertation is extensive and advances knowledge of reproductive physiology of broiler breeder hens. A major technical contribution was the development of cannulation procedures to allow successful collection of hourly blood samples for up to 240 h without affecting egg production. Conducting this research was time consuming. Without help, support, and encouragement from several persons, I would not have been able to complete this work.

First of all, I would like to express my deep gratitude and appreciation to my advisor, Dr. Wayne L. Bacon, for his excellent guidance, support, patient, enthusiastic encouragement, and invaluable comments throughout my master and doctoral studies. I have enjoyed every moment that we have worked together from hourly blood collections to data discussion and manuscript preparation.

My thanks also go to my committee members, Drs. Karl E. Nestor, Michael S. Lilburn, and Joseph Ottobre for reading manuscripts and the dissertation, and providing many valuable comments to improve their quality.

Special acknowledgement is given to Dr. David W. Long for his pleasant help and excellent technical assistance even though he has retired. I appreciate and thank Mr. John W. Anderson and Bijula Koyyeri for assisting with blood sample collection. I will also give a special thanks to the poultry farm crew for their help to record egg production
and caring for chickens during the research period, especially for Dennis Hartzler, Keith Patterson, and Jack Sidle.

Many thanks are also extended to Dr. John Proudman for providing LH reagents, Dr. John P. McMurtry for conducting the leptin and insulin assays, and Dr. Shuen-Ei Chen for providing leptin and insulin information. My thanks also to Mr. Chi-Wei Tsai and Mrs. Karen Tsai for friendship and having parties regularly to relax my life. I want to specially thank the department and OARDC for providing financial support to me and for my research.

Last, I especially thank my wife, LingBei, for her understanding, support, and love during the past few years, and my daughter, Emily, who makes my life joyful. I would like to thank my parents, and sisters for their life-long love and support. Without them, I could not have gotten this far, and this big project could not have been completed. To all of you, thank you.
VITA

November 28, 1971..............................................Born in Taichung, Taiwan

June, 1994..................................................B.S., Animal Sciences,
TungHai University, Taiwan, R.O.C.


1997-2000 ..................................................M.S. Animal Sciences,
The Ohio State University

2000-present..............................Graduate Research Associate
The Ohio State University

RESEARCH GRANTS

2000  Frequency of preovulatory luteinizing hormone surges may control egg
production rate in turkey hens
Ohio Agricultural Research and Development Center (OARDC) Research
Enhancement Competitive Grant ($2,500)

2002  Evaluating frequency of preovulatory luteinizing hormone surges in full-fed and
restricted-fed broiler breeder hens and their reproductive performance
Ohio Agricultural Research and Development Center (OARDC) Research
Enhancement Competitive Grant ($5,000)

ACADEMIC SERVICE

Manuscript Review for Biology of Reproduction


FIELD OF STUDY

Major Field: Animal Sciences
Specialty: Reproductive Physiology
# TABLE OF CONTENTS

Abstract ....................................................................................................................... ii

Dedication .................................................................................................................... v

Acknowledgments...................................................................................................... vi

Vita ........................................................................................................................... viii

List of Tables ............................................................................................................ xiv

List of Figures ......................................................................................................... xvii

Chapters:

1. Literature reviews ............................................................................................1
   Statement of the problem .................................................................................1
   Rationale and significance ..........................................................................4
   Cannulation procedures for serial blood sampling ...................................8
   Effects of feed consumption (ad libitum- vs restricted-fed) on female reproductive performance .................................................................9
   Luteinizing hormone (LH) surges, ovulation, and oviposition ................16
   Self-association and raft localization of functional luteinizing hormone receptors .........................................................................................21
   Effect of Photostimulation on secretion of LH ...........................................23
   Progesterone (P₄) .........................................................................................25
   Estradiol-17β (E₂) .....................................................................................28
   Follicle stimulating hormone .................................................................29
   Historical background information of leptin ...........................................31
   Leptin gene .................................................................................................33
   Leptin receptor ............................................................................................35
   Effect of administration of leptin on physiological responses ..........36
   Relationships between leptin and feeding treatments .........................38
   Effect of leptin injection on food intake, energy metabolism, and body weight .................................................................40
   Rhythm of peripheral leptin concentrations ............................................42
Leptin resistance ................................................................................44
Leptin and reproduction .....................................................................45
Peripheral concentrations of insulin and glucose and feeding
treatments ..........................................................................................48
Follicular development and postovulatory follicles associated with
unreconciled ovulation ......................................................................55
Number of yolky follicles and egg production ..................................57
Effects of feeding regimes, hen age, and follicle stimulating
hormone (FSH) on follicular growth ................................................59
Body weight and age at onset of sexual maturity ..............................61
Photostimulation and egg production ..............................................63
Hen age at photostimulation ............................................................64
Nutrient requirements ........................................................................65
Management of commercial breeder hens .......................................69

2. Development of a Cannulation Procedure for Broiler Breeder Hens ....71
   Abstract ..........................................................................................71
   Introduction ......................................................................................72
   Material and methods ......................................................................74
   Results ............................................................................................80
   Discussion ......................................................................................82
   References ....................................................................................85

3. Preovulatory surge patterns of luteinizing hormone, progesterone, and
   estradiol-17β in ad libitum-fed and restricted-fed broiler breeder hens .....94
   Abstract ..........................................................................................94
   Introduction .....................................................................................95
   Material and methods .....................................................................99
   Results ..........................................................................................101
   Discussion .....................................................................................103
   References ...................................................................................107

4. Preovulatory luteinizing hormone surge interval of broiler breeder hens
   increases with duration of the reproductive period but is not changed by
   feed restriction ................................................................................116
   Abstract ..........................................................................................116
   Introduction .....................................................................................118
   Material and methods .....................................................................122
   Results ..........................................................................................126
   Discussion .....................................................................................130
   References ...................................................................................139

5. Peripheral concentrations of leptin and insulin were not changed with
duration of the reproductive period but insulin concentration was lowered
in ad libitum-fed broiler breeder hens .............................................162
   Abstract ..........................................................................................162
6. Duration of follicular development increases with advancing duration of the reproductive period in broiler breeder hens

List of References
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Summary of cannulation system results</td>
<td>89</td>
</tr>
<tr>
<td>2.2</td>
<td>Distribution of when cannula patency was lost during 10 d of serial bleeding (hourly samples for 10 d) in Experiment 2</td>
<td>90</td>
</tr>
<tr>
<td>2.3</td>
<td>Distribution of when hens stopped laying (d of last oviposition) after cannulation or during serial bleeding in Experiment 2</td>
<td>91</td>
</tr>
<tr>
<td>2.4</td>
<td>Egg production (n) of the hens that did not stop laying for the 10 d before cannulation and the 10 d during serial bleeding of broiler breeder hens in Experiments</td>
<td>92</td>
</tr>
<tr>
<td>3.1</td>
<td>Body weight and egg production of ad libitum-fed (F) and restricted-fed (R) broiler breeder hens</td>
<td>112</td>
</tr>
<tr>
<td>3.2</td>
<td>Concentrations of luteinizing hormone (LH), progesterone (P₄), and estradiol-17β (E₂) early in the reproductive period of ad libitum (F) and restricted-fed (R) broiler breeder hens</td>
<td>113</td>
</tr>
<tr>
<td>3.3</td>
<td>Duration of preovulatory surges of luteinizing hormone (LH), progesterone (P₄), and estradiol-17β (E₂) early in the reproductive period of ad libitum (F) and restricted-fed (R) broiler breeder hens</td>
<td>114</td>
</tr>
<tr>
<td>4.1</td>
<td>Hen number, age, duration of production, and feeding treatments for the 6 trials</td>
<td>145</td>
</tr>
<tr>
<td>4.2</td>
<td>Main effect means±SE for body weight, total eggs laid the 10 d before cannulation and during the 10 d hourly blood sampling, and settable and abnormal egg production during the 10 d of hourly blood sampling</td>
<td>146</td>
</tr>
<tr>
<td>4.3</td>
<td>Interaction means±SE for body weight, total eggs laid the 10 d before cannulation and during the 10 d hourly blood sampling, and settable and abnormal egg production during the 10 d of hourly blood sampling</td>
<td>147</td>
</tr>
<tr>
<td>4.4</td>
<td>Main effect means±SE for luteinizing hormone (LH) concentration, LH</td>
<td>147</td>
</tr>
</tbody>
</table>
baseline, and surge amplitude concentrations, LH surge interval, percentage LH blind surges, progesterone (P4) baseline and surge amplitude concentrations, and estradiol-17β concentration (E2) in broiler breeder hens early and late in the reproductive period and among feeding programs ...........148

4.5 Interaction means±SE for luteinizing hormone (LH) concentration, LH baseline and surge amplitude concentrations, LH surge interval, percentage blind LH surges, progesterone (P4) baseline and surge amplitude concentrations, estradiol-17β concentration (E2), and estimated egg production rate of broiler breeder hens early and late in the reproductive period and among different feeding programs ...............................................149

4.6 Correlation coefficients (r) and statistical probability of significance (P) among duration of reproduction, LH baseline and LH amplitude (amp.) concentrations, interval between LH surges, percentage LH blind surges, P4 baseline and P4 amplitude concentrations, and overall mean E2 concentrations in broiler breeder hens ..........................................................150

5.1 Main effect means±SE for body, liver and fat pad weights, LH surge interval, and concentrations of leptin and insulin in broiler breeder hens early (Early) and late (Late) in the reproductive period and among feeding programs .....184

5.2 Interaction means±SE for body, liver and fat pad weights, LH surge interval, and concentrations of leptin and insulin, and coefficients of variation (CV) of leptin concentrations in broiler breeder hens early (Early) and late (Late) in the reproductive period ........................................185

5.3 Correlations (r) and statistical probability (P) among body weight, LH baseline concentration, LH amplitude (amp.) concentration, LH surge interval, estradiol-17β (E2) concentration, liver and fat pad weights, leptin and insulin concentrations, and duration of the reproductive period ..........186

6.1 Hen number, age, duration of production, and feeding treatments early (Early) and late (Late) in the reproductive period for the 6 trials .............222

6.2 Mean interval between luteinizing hormone (LH) surges, mean duration of follicular development during 10 d of serial bleeding, and number of hierarchical follicles in each feeding group early and late in the reproductive period .................................................223

6.3 Mean interval between luteinizing hormone (LH) surges during 10 d of serial bleeding and numbers of hierarchical follicles, identical sequence, multiple hierarchical follicles, and follicular development stages in each feeding group ........................................................................224

6.4 Main effect means±SE for body, ovary and oviductal weights, number of
atretic and hierarchical follicles, F1 follicle weight, identical dye ring sequences, multiple hierarchical follicles, follicular development stages, follicular development, and rest period in broiler breeder hens early (Early) and late (Late) in the reproductive period and among feeding programs .......................................................................................................226

6.5 Interaction means±SE for body, ovary and oviductal weights, number of atretic and hierarchical follicles, F1 follicle weight, identical dye ring sequences, multiple hierarchical follicles, follicular development stages, follicular development, and rest period in broiler breeder hens early (Early) and late (Late) in the reproductive period and among feeding programs .....228

6.6 Correlations (r) and statistical probability (P) in broiler breeder hens .......230
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Patterns of the duration of egg production reported in various studies</td>
<td>70</td>
</tr>
<tr>
<td>2.1</td>
<td>Cannulation in laying broiler breeder hens</td>
<td>93</td>
</tr>
<tr>
<td>3.1</td>
<td>Peripheral patterns of luteinizing hormone (LH), progesterone (P₄), and estradiol-17β (E₂), in plasma of ad libitum-fed or restricted-fed broiler breeder hens at 12 min (LH), 24 min (P₄), or 3 h (E₂) interval for 36 h.</td>
<td>115</td>
</tr>
<tr>
<td>4.1</td>
<td>Hen d⁻¹ % egg production rate of ad libitum-fed (FF), ad libitum-fed switched from restricted-fed (RF), and restricted-fed (RR) broiler breeder hens</td>
<td>151</td>
</tr>
<tr>
<td>4.2</td>
<td>Hourly plasma concentrations of luteinizing hormone (LH) for 240 h in representative broiler breeder hens (data presented are for hens with mean surge interval closest to mean of each experimental group).</td>
<td>152</td>
</tr>
<tr>
<td>4.3</td>
<td>Distributions of all LH surge intervals of FF, RF, and RR hens early (a) or late (b) in the reproductive period.</td>
<td>159</td>
</tr>
<tr>
<td>4.4</td>
<td>Distributions of hen mean LH surge intervals of FF, RF, and RR hens early (a) or late (b) in the reproductive period.</td>
<td>160</td>
</tr>
<tr>
<td>4.5</td>
<td>Egg production in broiler breeder, turkey, and Leghorn hens.</td>
<td>161</td>
</tr>
<tr>
<td>5.1</td>
<td>Hourly plasma luteinizing hormone (LH), and leptin and insulin concentrations every 4 h for 240 h in representative broiler breeder hens.</td>
<td>187</td>
</tr>
<tr>
<td>6.1</td>
<td>Dye ring sequence patterns produced by daily feeding of either Sudan IV (R) or Sudan Black B (B) to broiler breeder hens.</td>
<td>231</td>
</tr>
<tr>
<td>6.2</td>
<td>The relationship between duration of follicular development and oviposition during 10-d periods.</td>
<td>232</td>
</tr>
</tbody>
</table>
CHAPTER 1

LITERATURE REVIEWS

Statement of the problem:

The strong negative association between growth rate (mature body weight) and egg production rate in turkeys (Nestor et al., 1996; Liu et al., 2001c) and chickens (Grossman et al., 2000; Nøddegaard et al., 2000) has been clearly illustrated. Egg production rate of chicken and turkey hens sharply increases to a peak a few weeks (depending on feeding program and species) after photostimulation. Peak production is following by a period of persistent high production for several weeks and then a period of gradual seasonal decline as the reproductive period progresses (Figure 1.1). Egg production is hormonally controlled but what hormonal factors are associated with egg production rate during the peak and seasonal decline portions of a reproductive period is the major research issue in our laboratory. It was recently reported that Egg line turkey hens with excellent egg production rate (peaked at 85%) but light body weight (6.5 kg)
had a shorter interval between luteinizing hormone (LH) surges at peak production in comparison to a line (RBC3) with large mature body size (13kg) but poor egg production rate (peaked at 65%) (Anthony et al., 1991; Noble et al., 1995; Liu et al., 2001c). Leghorn laying chickens with small mature body weight (2 kg) have been selected for increased egg production rate. Broiler breeder chickens with large mature body weight (5 kg) have been selected for rapid growth rate. In commercial practice, restricted-feeding is used because it results in increased egg production and settable eggs in broiler breeder hens. In broiler breeder hens, selection for a more rapid growth rate has resulted in poor egg production in comparison to strains of chicken hens selected for increased egg production. The pattern of egg production rate between Leghorn chickens with longer duration of production and restricted-fed broiler breeder chicken with shorter duration of production is very similar to small body (Egg line) and large bodied (RBC3 line) turkeys (Figure 1.1).

Most commercial broiler breeder hens are feed-restricted to limit body weight during rearing or breeding. In restricted-fed broiler breeder hens, body weight and age of initiation of egg production are more uniform in comparison to ad libitum-fed hens. The difference in the pattern of egg production between ad libitum-fed and restricted-fed
broiler breeder hens is similar to that of ad libitum-fed large and small bodied turkeys when they start laying (Figure 1.1). Our recent study with turkey hens (Liu et al., 2001c) showed that at peak of production, egg production rate is lower for large bodied (RBC3) hens, is maintained for a shorter duration, and declines faster than for small bodied (Egg line) turkey hens (Figure 1.1). The increased egg production in restricted fed broiler breeders is due to a physiological change associated with restricted feeding. The physiological basis for this increased female reproductive performance induced by restricted feeding is however, not well understood. In broiler breeder hens, egg production in ad libitum-fed hens (about 5.5 kg) starts about 2 to 3 wk after photostimulation at 21 wk of age whereas egg production starts about 6 wk after photostimulation in restricted-fed broiler breeder hens (about 3.3 kg). Thus, egg production in restricted-fed broiler breeder hens starts about 3 wk later than in ad libitum-fed hens (Noddegaard et al., 2000). It reaches a higher peak of production in restricted-fed hens, is maintained longer, and then drops at a slower rate than in ad libitum-fed broiler breeder hens. This results in greatly improved egg production in restricted-fed broiler breeder hens over a 40-wk reproductive period, which is standard for broiler breeder hens. The goal of this study was to develop a better physiological
understanding of why the peak egg production rate of broiler breeder hens restricted-fed during rearing and laying is greater than for hens ad libitum-fed during rearing and laying. Physiological measurements included measuring concentrations and surge patterns of hormones related to reproduction, especially the intervals between luteinizing hormone (LH) and progesterone (P₄) surges that are necessary to induce ovulation of mature hierarchical follicles. The objective of this study was to measure the interval between and characteristics of preovulatory surges of LH and P₄, and concentrations of estradiol-17β (E₂), leptin, and insulin early and late, when egg production had declined 25% compared to early production, in the reproductive period in hens either restricted-fed (RR, projected high egg production rate), ad libitum-fed (RF) switched from restricted-fed when photostimulated, or ad libitum-fed (FF) projected low egg production rate.

**Rationale and Significance:**

**Rational:**

Egg production rate in restricted-fed broiler breeder hens has been studied through the entire reproductive period, but limited data are available for comparison with
ad libitum-fed hens. Ad libitum-fed broiler breeder hens initiate the onset of sexual maturity with a large variance about 2 wk after photostimulation. A greater number of ovarian yolky follicles and poorer egg production rate were found in ad libitum-fed broiler breeder hens in comparison to restricted-fed hens (Yu et al., 1992b; Noddegaard et al., 2000). In turkey hens, Liu et al. (2001c) reported intervals between preovulatory surges of LH and P₄ were a major factor contributing to the difference in egg production rate early in the reproductive period from lines with a known difference in egg production. A second factor was an increased incidence of blind surges of LH. A preovulatory surge of LH not retrospectively associated with oviposition of an egg is called a blind surge (Liu et al., 2001a, b, c). The longer intervals between surges of LH and P₄ were associated with poor egg production rate in a large bodied line of turkeys (RBC3) and shorter surge intervals were associated with excellent egg production rate in a small bodied line of turkeys (Egg) selected for increased egg production. However, the basic relationships of preovulatory surges of reproductive hormones and ovulation/oviposition rate have not been studied in detail in ad libitum-fed and restricted-fed broiler breeder hens, though ad libitum-fed broiler hens and large bodied turkey hens are known to have a larger and a greater number of ovarian follicles and poorer reproductive potential (Yu et
al., 1992b; Liu et al., 2000c). It was hypothesized at least three primary causes of poor egg production in ad libitum-fed broiler breeder hens. First: a greater interval between LH surges and ovulations in ad libitum-fed hens. A mature follicle can be ovulated only when it receives the signal of a preovulatory surge of LH secreted by the anterior pituitary, but immature follicles do not respond. Second: a higher incidence of blind LH and P₄ surges may occur in ad libitum-fed hens, resulting in a greater interval between eggs leading to fewer eggs during a reproductive period. Third: a combination of longer interval between surges of LH and a higher incidence of blind surges of LH may be involved.

**Significance:**

In Ohio, the poultry industry accounts for $650 million annually. The poultry industry provides humans a good source of high quality protein both as eggs and meat at competitive prices. In the US poultry industry, consumption of broiler chicken and turkey meats is still increasing year by year. For broiler chicken, the production increased from about 3.1 billion birds in 1980 to 8 billion in 2000. Each broiler breeder hen can produce 125 chicks in a year. So we can estimate that about 65 million broiler
breeder hens will be needed in one year. Turkey production increased from about 150 million birds in 1980 to 230 million in 2000. Each turkey breeder hen can produce about 70 turkey poults. It can be estimated that about 3.2 million turkey hens will be needed each year. The data show that about 20 times more broiler breeder hens are needed each year than turkey breeder hens. Increasing growth rate and associated feed efficiency in modern broiler chickens have been achieved by genetic selection and improved nutrition. However, a negative association of egg production is coupled with the rapid growth rate of modern broilers. Although female reproductive efficiency can be improved by restricted feeding during growth and reproduction, the basic physiological mechanism of this increase remains unknown. The aim of this study was to discover if either the interval between preovulatory surges of LH and P₄, the incidence of blind surges, a combination of these two factors, peripheral concentrations of E₂, leptin, and insulin, or some yet to be discovered factor is the source of the negative association between growth rate and female reproductive efficiency in modern broiler breeder hens.
Cannulation procedures for serial blood sampling:

To monitor the secretion pattern of reproductive hormones in vivo, one needs proper cannulation procedures to allow for long-term serial blood sampling in unrestrained and unstressed birds. Hourly serial blood sampling of turkeys has been successfully developed for long-term blood sampling (up to 13 days) in the author’s laboratory (Chapman et al., 1994; Yang et al., 1997, 2000; Bacon et al., 2002; Liu et al., 2001a, b, c, 2002). However, no studies have been reported where long-term serial blood sampling of chicken hens has been used to estimate secretion patterns of reproductive hormones, due to the technical problems associated with collecting hourly serial blood samples from unrestrained and unstressed hens. Thus, the peripheral patterns of LH, P₄, E₂, leptin, and insulin have not been measured during multiple ovulatory cycles of individual chicken hens. Recently, Liu et al. (2004a) have addressed the technical problems associated with long-term cannulation of broiler breeder hens for serial blood sample collection. The procedures previously used in turkey hens were successfully modified so that the cannulas of cannulated laying broiler breeder hens, either ad libitum-fed or restricted-fed, remained patent for at least 4 wk and the hens kept
laying during a serial bleeding trial. These procedures are described in detail in Chapter 2 of this dissertation.

**Effects of feed consumption (ad libitum- vs restricted-fed) on female reproductive performance:**

Feed restriction is a standard practice in broiler breeder management and it has been shown to be an efficient and economical way to achieve better female reproductive efficiency (Robinson et al., 1991; Yu et al., 1992a). Increased female reproductive efficiency, however, was not found in restricted-fed turkeys compared to ad libitum-fed (Hester and Stevens, 1990; Crouch et al., 1999). Various methods of physical feed restriction have been used: 1) skip 1d (Wilson et al., 1983; Lesson and summers, 1985; Wilson et al., 1989; Ballay et al., 1992), skip 2 d (Bartov et al., 1988), and daily feed restriction (Lesson and Summers, 1985; Lee, 1987; Wilson et al., 1989). Gous et al. (2000) reported that both light and feed restriction have an effect on age at sexual maturity of laying hens. Cave (1984) and Brake et al. (1985) reported that nutrient intake immediately before the onset of sexual maturity was the most important factor to
influence the laying performance of broiler breeders. Their studies suggested that a high protein diet increased egg size and improved egg production.

The study of Summers and Lesson (1994) showed that although broiler breeders fed two lower protein growing diets (11 or 14 %) resulted in lower egg production during the first 4 wk of egg production, egg production was not different by 28 wk of age among treatments (hens fed diets containing 20, 17, 14, or 11 % protein with similar energy content of approximately 2,950 kcal ME/kg of diet between day-old and 16 wk of age). Recent studies of Bruggeman et al. (1997, 1998, 1999) in restricted-fed broiler hens showed that a change in plasma metabolic hormones occurred, from 2 to 7 wk, as an effect of restricted feeding compared to ad libitum feeding. The period around Week 16 appeared to be important for initiating the development of reproductive functions in broiler breeder females. Bruggeman et al. (1999) reported that restricted feeding of broiler breeders from 7 to 15 wk of age was associated with higher proportional weights of ovary and oviduct at the age of sexual maturity. The data also have shown that restriction level during the second (wk 7 to 15) and third (wk 16 to first egg) period is a more important determinant of age or body weight at first oviposition, but not the first period (wk 2 to 6) of restriction. Yu et al. (1992b) reported that controlled feeding and
growth from 4 to 18 wk is more important to determine age at sexual maturity than feed intake and change in growth rate from wk 18 until onset of laying. Pym and Dillion (1974) suggested that severe restricted feeding during rearing and switching to ad libitum feeding might contribute to better egg production in broiler breeders.

Egg production and average egg weight were not different when broiler breeder hens were restricted-fed for a 4 wk period after 56 wk of age (Goerzen et al, 1996). The study has shown that the incidence of multiple ovulations was a major source of lost egg production and that restricted feeding should be applied until the onset of lay (Hocking, 1993). During the rearing period, limiting body weight gain by restricted feeding was applied to delay sexual maturity and usually resulted in a longer duration of peak egg production in broiler breeder females. The longer duration of peak egg production did not apply in turkeys (Crouch et al., 1999), however, body weight has been reported to be a critical parameter for an effective restricted feeding program (Luther et al., 1976). The study suggested that a minimum body weight and chronological age might be a requirement for sexual maturity in broiler breeder females (Fattori et al., 1993). Feed intake and growth during the rearing period were important to determine the age at sexual maturity in broiler breeders (Yu et al., 1992a). Robinson et al. (1996) found that the
coefficient of variability (CV) of age and body weight at the first egg decreased as the age of photostimulation increased in feed restricted broiler breeders. Restricted fed broiler breeders had a delay in the onset of laying, and a more synchronous (90% of birds) entry into laying within 18 d in comparison to 49 d for ad libitum-feed hens (Eitan et al., 1998). Similar results were found by Hocking et al. (1994); the age at the first egg was delayed by about 2 wk in restricted-fed hens, but at 54 wk of age they had laid more eggs (114 compared to 67 eggs). In summary, egg production pattern and rate can be modified by feed restriction programs and lighting stimulation program.

Broiler breeder females are at a reproductive disadvantage when they are allowed to ad libitum-feed (Jaap and Muir, 1968; Hocking et al., 1987a; Robinson et al., 1993). Excess body weight and especially body fat may be involved in reducing reproductive efficiency (McDaniel et al., 1981; Pearson and Herron, 1981). The inefficiency of reproduction includes: reduced egg production rate (McDaniel et al., 1981; Robinson et al., 1991), poorer shell quality, increased incidence of multiple-yolked eggs, erratic timing of ovipositions (Jaap and Muir, 1968), obesity-related mortality (Tottori et al., 1997), short duration of fertility (Goerzen et al., 1996), and increased embryonic loss (Yu et al., 1992a). However, Chany and Fuller (1975) reported that obesity had no
significant effect on egg production of broiler breeders. A few studies showed that egg production was improved by ad libitum feeding during part or all of laying period (Pym and Dilon, 1974; Robbins et al., 1986, 1988). Bruggeman et al. (1999) reported that pullets fed ad libitum had the heaviest body weight, the youngest age at first oviposition, but a small proportional ovary and oviduct weight whereas restricted feeding resulted in reduced body weight, delayed age of sexual maturity, and a higher proportionally in ovary and oviduct weight. However, egg production is multi-factorial in broiler breeder hens.

An increasing incidence of short laying sequences and number of pause days in broiler breeders was observed when they were switched to ad libitum feeding from restricted feeding at the onset of sexual maturity (Robinson et al., 1991). The mean prime laying sequence (longest sequence) from 24 to 62 wk of age was 14.9 d for ad libitum-fed hens compared to 24.9 d for restricted-fed hens (Robinson et al., 1991). The reduced reproductive performance induced by ad libitum feeding was associated with a decrease in the length of the laying sequence and an increase of the number of pause days. Robinson et al. (1996) reported that ad libitum-fed hens had a greater number of laying pauses of greater than 11 d, which they suggested was associated with ovarian
regressions followed by resumption of laying. Recent studies (Liu et al., 2001c) in laying turkey hens found that the incidence of blind surges of LH was one factor associated with decreased egg production in a large bodied line. Whether the large number of longer pause days is due to a higher incidence of blind surges of LH in broiler breeders needs to be further studied. In summary, lower egg production is associated with shorter laying sequences and more pauses (>11d) in ad libitum-fed in comparison to restricted-fed broiler breeder hens.

In ad libitum-fed broiler breeder chickens, several factors may contribute to poorer egg production early in the reproductive period. An increase in the interval between intra-sequence LH surges leads to longer intervals between ovipositions and results in poorer egg production (Lillpers and Wilhelmson, 1993). Recent studies in turkeys with different rates of production showed that a longer interval between preovulatory surges of LH and P₄ was a major factor associated with poor egg production rate at peak production (Liu et al., 2001c). An increase in the incidence of blind LH surges was also associated with poor egg production (Liu et al., 2001c). A possible explanation for a blind surge is that a mature ovarian follicle is stimulated to ovulate by an LH surge but the ovulated ovum does not enter the infundibulum to be formed into a
completed egg, resulting in an internal ovulation. Causes of internal ovulations are largely unknown but may be due to abnormal or lack of muscular contraction of the infundibulum. A higher incidence of interval ovulation was reported in commercial male (Sire) line turkey hens with a rapid growth rate at the onset of lay in comparison to female (dame) line (Melnychuk et al., 1997).

An increase in the incidence of inter-sequence intervals of longer than 33 h is also associated with a poor egg production rate (Yu et al., 1992b). Inter-sequence intervals occur more frequently late in a reproductive period and are associated with pauses between clutches of eggs. Ad libitum-fed broiler breeder hens have more inter-sequence pauses resulting in poorer egg production rate (Yu et al., 1992b). A longer duration of follicular development with greater number of hierarchical follicles has also reported for ad libitum-fed broiler breeder hens (Yu et al., 1992b), possibly resulting in a longer interval between LH surges, was associated with poor egg production. A loss of synchronization of ovulation and oviposition (Yu et al., 1992b), resulting in an increase in defective eggs, was associated with poor egg production in ad libitum-fed broiler breeder hens. Also abnormal secretion of P₄, associated with a
blockage of LH surge secretion and oviposition in laying turkey hens (Liu et al., 2001b),
was associated with poor egg production in this species.

Luteinizing hormone (LH) Surges, Ovulation, and Oviposition:

Ovulation and oviposition are hormonally controlled process in birds. Surge
secretion of LH is the major signal to induce spontaneous ovulation of mature
hierarchical follicle (s) in birds, and has been extensively studied in laying turkeys in Dr.
Bacon’s laboratory (Chapman et al., 1994; Yang et al., 2000; Liu et al., 2001a, b, c).
Exogenous injection of LH in acutely hypophysectomized laying chicken hens induces
single or multiple ovulations of mature hierarchical follicles in in vivo studies (Opel and
Nalbandov, 1961a, b). In birds, secretion of preovulatory surges of LH from the
anterior pituitary is stimulated by gonadotropin releasing hormone-I by the hypothalamus
as in mammals.

High resolution patterns of secretion of LH have been determined for
photosensitive, photostimulated (before and after first egg) laying, and forced molted
turkey hens, and the following characteristics of secretion noted (Chapman et al., 1994;
Bacon and Long, 1995, 1996): 1) In photosensitive female turkeys, the secretion of
pulsatile LH is characterized by relatively low overall and baseline LH concentrations with well-defined LH pulses of relatively low frequency, short duration, and high amplitude (Bacon and Long, 1995). Pulses of LH in photosensitive turkey hens are characterized by a sharp increase to a well defined peak and a slow decline to baseline level. Duration of these surges is < 1.0 hr (Bacon and Long, 1995; Yang et al., 1997).

2) After photostimulation of photosensitive hens, the baseline concentration of LH increased during the first long-day scotoperiod, after the first long-day photoperiod with a further increase during the second long-day scotoperiod (Bacon and Long, 1995). Thus, the response to photostimulation is relatively rapid.

3) After several days of photostimulation but before initiation of egg production, the secretion of LH is characterized by a relatively high baseline concentration, with a few low amplitude pulses (Chapman et al., 1994). The high baseline concentration was maintained until the onset of egg production.

4) When egg production starts, the dominant feature of LH secretion becomes preovulatory surges of LH superimposed and nearly constant high baseline concentration of the hormone (Chapman et al., 1994). In turkey hens, the secretion pattern of preovulatory LH surges is characterized by an increase in concentration to a peak over 1 to 2 h followed by a slow decline to baseline level over 3
to 5 h (Chapman et al., 1994; Yang et al., 1997), a much different pattern than seen in photosensitive hens. In photosensitive hens, pulses occurred every 2 to 3 hr, while in laying hens, surges occurred every 24 to 36 h (Chapman et al., 1994; Liu et al., 2001a, b, c).

5) As the reproductive period progressed, the interval between LH surges increased but LH baseline concentrations declined (Liu et al., 2002).

Spontaneous ovulation of the largest mature follicle(s) occurred within 30 min after a preceding oviposition in the laying sequence of turkey hens (Wolford et al., 1964). Early in the egg production period oviposition can be calculated to occur approximately 34 h subsequent to the peak of an LH surge when the interval between surges of LH is 26 h (Liu et al., 2001c). Each oviposition was coincident with a preceding preovulatory surge of LH during the laying period (Yang et al., 2000), but the preovulatory surges of LH were not always coincident with ovipositions. This results in the interval of ovipositions not always being the same as the interval between surges of LH. The uncoupled preovulatory surges of LH were called blind surges (Liu et al., 2001 a, c), suggesting the infundibulum of the oviduct failed to pick up an ovulated ovum or that ovulation failed to occur. A possible explanation is that an ova is ovulated but not picked up by the oviduct, resulting in internal ovulations with the yolk reabsorbed in the
body cavity. A high incidence of blind surges during ovulatory cycles can contribute to poor egg production during an egg production period (Liu et al., 2001a, c). However, the incidence of blind surges of LH was not different between young turkey hens early in the laying period and old hens late in the laying period (unpublished data), indicating that within strain the incidence of blind LH surges does not change as the hen age.

A longer interval between surges of LH contributed to poorer egg production in large bodied RBC3 hens (Liu et al., 2001c) in comparison to small bodied Egg line hens. A recent study in turkeys at the onset of egg production showed in arrested laying hens that a hard shelled egg may be held in a hen’s shell gland for a long time (over 2 wk) instead of spontaneously oviposited. This occurred when concentrations of progesterone were maintained at a relatively high level of 5.0 ng/ml (Liu et al., 2001b). Direct evidence has shown in laying turkey hens that high incidence of polycystic ovarian follicle syndrome (PCOF) and holding hard shell egg in uterus was induced by daily exogenous injection of P₄ (>0.33 mg/kg/d) for 2 wk (Bacon and Liu, 2004). These results suggested that continuously high concentrations of progesterone might inhibit secretion of LH, block oviposition, but not disrupt the entrance into, or development of hierarchical follicles, resulting in a PCOF (Liu et al., 2001b; Bacon and Liu, 2004).
Over 30 yolky follicles have been observed in turkey hens with this PCOF. In one case, an arrested laying hen died when all developing ovarian follicles (type I, II, and III) collapsed in the body cavity after photostimulation at an early age (i.e., 30 wk of age). Poor reproduction may be due to an increased incidence of blind surges of LH and P₄, or an arrest in laying early in the reproductive period, such as occurs at a higher frequency in young turkey hens photostimulated before 30 wk of age.

The reduced LH pulse frequency was associated with the slow frequency of gonadotropin releasing hormone (GnRH) release when sheep were restricted-fed (I’Anson et al., 2000; Nagatani et al., 2000). Pulsatile secretion of LH was elevated when the animals were subsequently refed (Monkey: Schreihofer et al., 1993; Lamb: Foster and Olster, 1985). The decreased in frequency of LH pulses was prevented after leptin treatment in fasting sheep (Nagatani et al., 2000), rats (Nagatani et al., 1998), and monkeys (Finn et al., 1998). Increased frequency of LH pulses was not only found in fasting animals treated with leptin, but it was also found that in nonfasted, ad libitum-fed rats administrated leptin the LH pulse amplitude was increased (Yu et al., 1997a).
Self-association and raft localization of functional luteinizing hormone receptors:

Membrane microdomains called lipid rafts are enriched in cholesterol and sphingolipids (Brown and London, 2000; Mayor and Rao, 2004). Lipid rafts have been postulated to be the signaling center for G-protein interaction. Many studies have shown that plasma receptors, including IgA and epithelial growth factor receptors, moved to membrane rafts during signal transduction. Dr. Roess and her colleague (Hunzicker-Dunn et al., 2003; Roess and Smith, 2003) used a variety of techniques to examine the interactions between LH receptors and ligands on plasma membrane and tried to identify whether membrane microdomains (lipid rafts) are involved in the interaction between LH and its ligand receptor.

The LH receptor is one of the membrane receptors with signal transduction involving G proteins to stimulate the production of cAMP when functional LH receptor binds hormone agonists [i.e. LH or human chorionic gonadotropin (hCG)]. To determine whether functional LH receptors exhibit slow rotational diffusion in membranes, rotational dynamics were compared using time-resolved phosphorescence anisotropy methods. The rotational diffusion of membrane proteins is linearly related to the in-membrane volume of the complex identified by the phosphorescent probe (Roess et al.,
2000). The results have shown that functional LH receptors had longer rotational correction times than nonfunctional receptors, suggesting functional receptors had slow diffusion in membranes. These data indicate that upon binding of agonist and activation, LH receptors are incorporated into lipid rafts.

Fluorescence-resonant energy transfer studies have shown that functional and desensitized LH receptors, induced by incubation with guanosine triphosphate (GTP), were associated with self-association. LH receptors exhibit desensitization by 1 h in response to saturating hormone concentrations (Horvat et al., 2001; Hunzicker-Dunn et al., 2003). Finally, they tested whether functional LH receptors with bound ligand were involved in membrane rafts by using Western blots. The results have shown that functional receptors together with caveolin, a protein that serves as a lipid raft membrane marker, were located at 12 to 25% sucrose after density gradient configuration whereas unoccupied LH receptors were found at 50 to 60% sucrose. These data suggest that functional LH receptors translocate into specialized membrane microdomains (rafts).

Taken together, functional receptors located on the plasma membrane were self-associated when bound to the ligands and the binding drove the receptors into membrane lipid rafts which induced the receptors to be more self-associated than active
receptors and to become desensitized. About 4 to 5 h after binding ligands, the LH receptors moved out of lipid rafts and became resensitized characterized by a reduction in receptor-receptor interaction and were capable of binding the ligands again.

**Effect of photostimulation on secretion of LH:**

Extraretinal photoreceptors in the hypothalamus receive the photo-signal that controls the secretion of GnRH-I into the hypothalamic-hypophyseal portal system in birds. The GnRH-I delivered to the anterior pituitary regulates the release of the gonadotropins into the systemic circulation. The surge release of the gonadotropins LH and possibly follicle stimulating hormone (FSH), probably stimulated by GnRH-I, induces ovulation of mature follicle(s). Photostimulation stimulates female reproductive functions whereas photorefractoriness results in the regression of reproductive functions in domestic hens (Sharp, 1993) and in other avian species (Sharp, 1996) under previously stimulatory photoperiods. Siopes (1998) reported that daily photoperiods exceeding 12 h will activate the process of photorefractoriness in turkey hens. The critical day length (CDL), the shortest duration long-day photoperiod for stimulation of egg production, was less than for photorefractoriness (11 to 11.5 and 12 to 12.5 h, respectively; Siopes, 1994).
Changes in the secretory pattern of LH associated with ovarian development occur after photostimulation of photosensitive turkey hens. Secretion of LH was characterized by low frequency, high amplitude pulses on a low baseline in photosensitive turkey hens (Chapman et al., 1994). Bacon and Long (1995) reported that LH concentrations increased during the first scotophase after the first day of Long Day (LD) photostimulation after switching photosensitive hens from Short Day (SD) to LD lighting. They measured LH secretion after photostimulation of photosensitive hens by serial bleeding every 15 min for 48 h. After photostimulation, the secretion pattern of preovulatory LH was characterized by a high baseline with few pulses of low amplitude and short duration (Chapman et al., 1994). The results suggested that a low amplitude, high frequency pattern of secretion of LH in plasma stimulated ovarian growth and gonadal steroid secretion (Chapman et al., 1994).

The preovulatory surge of LH is characterized by high baseline concentrations with short duration ascending limbs (2 to 3 h) and longer duration descending limbs (4 to 6 h), starting about 8 h before ovulation and lasting 6 to 8 h when the hens started to lay eggs, 2 to 3 wk after photostimulation (Yang et al., 1997, 2000). Ovulatory surges of LH only occurred when the mature follicles were present in the ovary (chicken, Etches,
Yang et al. (1997) reported that during ovulatory cycles the time of P₄ peaks was slightly earlier and lasted slightly longer than coincident LH peaks. Surges of LH and P₄ have been observed between 4 to 7 h before ovulation in laying hens (chicken: Furr et al., 1973; Senior and Cunningham, 1974, Shodono et al., 1975, Johnson, 1990; quail: Doi et al., 1980; duck: Tanabe et al., 1980; Wilson et al., 1982) and 2 to 8 h before ovulation in turkey hens (Mashaly et al., 1976; Proudman et al., 1984; Liu et al., 2001a, c). Baseline concentrations of LH gradually declined in laying hens as the laying period advanced (Guemene and Williams, 1994). Bacon and Long (1996) reported that basal LH concentrations were not different in laying hens at the end of a 40-wk period of egg production in comparison to hens force molted and given short day lighting for 7 wk.

**Progesterone (P₄):**

Progesterone has positive and negative effects on secretion of LH, on the ovaries to ability develop hierarchical follicles, and on ovulation/oviposition in birds (Liu et al., 2001b; Bacon and Liu, 2004). Administration of exogenous P₄ has been shown to induce premature ovulation of a mature follicle(s) at a specific time during ovulatory
cycles in normal laying chicken hens (Nakada et al., 1994). Progesterone is the major hormone secreted by granulosa cells of large mature hierarchical follicles in birds and thus may induce ovulatory surges of LH. High resolution data on secretion of LH and P₄ every 10 min for 26 hr has been reported during the ovulatory cycle in turkey (Yang et al., 1997), and every 12 min for 36 hr in chicken (Liu et al., 2004b). Preovulatory P₄ surges always increase at about the same time in relation to LH surges and are coincident with LH surges in turkey hens (Yang et al., 1997; Liu et al., 2001a, b, c, 2002; Bacon et al., 2002). But not all LH and P₄ surges are associated with specific ovipositions (Liu et al., 2001a, b, c; Bacon et al., 2002). Surges not associated with ovipositions were called blind surges (Liu et al. 2001a, b, c). A possible explanation of preovulatory blind surges of LH and P₄ is the occurrence of internal ovulations, where a mature follicle was ovulated but not picked up by the infundibulum to completely form an entire hard shelled egg. Also, high baseline concentrations of P₄ were associated with arrested laying and disrupted distribution of hierarchical follicles in turkeys (Liu et al., 2001b, Bacon and Liu, 2004). It is suggested that in arrested laying hens the high baseline concentrations of P₄ might negatively feedback on the ability of the hypothalamus to secret surges of GnRH.
and subsequently surges of LH, or on the ability of the pituitary to respond to surges of GnRH secretion if they occur (Liu et al., 2001b, Bacon and Liu, 2004).

The granulosa layer of larger mature follicles is the major site for production of P₄, whereas the theca layer of the smaller yolky follicles is the site for the synthesis of E₂ (chicken: Huang et al., 1979; Bahr et al., 1983; turkey: Porter et al., 1991). Increased secretion of P₄ was associated with the growth of follicles and the presence of mature yolky follicles (Etches et al., 1981; Porter et al., 1991; Liu et al., 2001b; Bacon et al., 2002). Exogenous injection of P₄ has been shown to arrest laying and induce retention of a hard shell egg in shell gland, and inhibit oviposition in turkeys (Bacon and Liu, 2004) and Japanese quail (Tell et al., 1999). Injection of P₄ has also been shown to induce preovulatory surges of LH in domestic fowl (Wilson and Sharp, 1975; Lang et al., 1984; Johnson et al., 1985) but not in ovariectomized chickens (Wilson and Sharp, 1976). Nakada et al. (1994) reported that exogenous injection of P₄ in hypophysectomized hens induced premature ovulation. An exogenous injection of P₄ did not induce surge-like secretion of LH before and at the start of laying in turkey hens (Yang et al., 1998).
**Estradiol-17β (E₂):**

Estradiol-17β is primarily secreted by theca cells of small ovarian follicles and stroma tissue, and is sensitive to LH and FSH stimulation in birds. In photosensitive turkey hens, plasma E₂ concentrations are at low levels (<0.1 ng/ml), but increase by 1 wk after photostimulation to between 0.3 and 0.5 ng/ml. After photostimulation of chickens and turkeys, the ovary and oviduct become active and start development due to E₂ stimulation. An increase in E₂ concentrations also induces the synthesis of yolk lipoprotein precursors by liver, which is necessary for ovarian follicle growth (Bacon, 1994; Chen et al., 1999).

Most E₂ is secreted by theca cells of small follicles and stroma tissue in domestic chicken hens (Bahr and Palmer, 1989; Nitta et al., 1991a, b). Baseline E₂ concentrations increased from < 0.1 ng/mL to 0.3 to 0.5 ng/mL 1 wk after photostimulation of hens, and is associated with development of the oviduct (Yang et al., 1999). One of the physiological functions of E₂ is to induce the synthesis and secretion of yolk lipoproteins by liver (Bacon 1994; Walzem, 1996; Chen et al., 1999). Treatment with E₂ can modify the concentration of cytoplasmic P₄ receptors in the reproductive tract (Pageaux et al., 1983). Plasma E₂ concentrations are maintained at very stable
concentrations the 4 d before first and after ovipositions begin in laying turkey hens (Bacon et al., 2002), suggesting spontaneous ovulations are not controlled by changing E₂ concentrations during ovulatory cycles. Plasma concentrations of triglyceride (TG) were associated with the change of the stage of reproductive performance in turkey hens. A high level of TG in laying hens was associated with the precursor synthesis of very low density lipoprotein (VLDL) and plasma transport to ovarian follicles (Bacon, 1994). Lower concentrations of TG were found in nonlaying, force molted hens, or males compared to laying hens (Bacon et al., 1980; Bacon and Long, 1996). Concentration changes in E₂ appear to be associated with reproductive functions other than the control of time of LH surges in chickens and turkeys.

**Follicle stimulating hormone (FSH):**

Follicle stimulating hormone is released from the anterior pituitary and is probably stimulated by GnRH-I from the hypothalamus in birds as in mammals. Palmer and Bahr (1992) reported that exogenous treatment with FSH stimulated the ovarian follicular recruitment into the hierarchy in chickens late in the reproductive period. No FSH concentrations can be measured in turkeys because of lack of purified turkey FSH
and a FSH antibody for radioimmunoassay. Chicken FSH assays have not been validated for turkey FSH. Plasma concentrations of FSH were not different between restricted-fed and ad libitum-fed broiler breeder hens when they were at 15 wk of age and at first egg, but were higher in ad libitum-fed hens than in restricted-fed hens at 18 wk of age (Bruggeman et al., 1999). No further evidence was found indicating higher plasma levels of FSH in ad libitum-fed broiler breeder hens may be associated with a greater number of yolky follicles in comparison to a lesser number of yolky follicles in restricted-fed hens.

The decline in egg production may be related to a decrease of FSH secretion, but egg production rate may not be related to FSH early in the reproductive period. New evidence, including changes of FSH concentrations via long-term hourly serial blood sampling during ovulation and ovulatory cycles should be obtained to clarify the relationship between number of ovarian hierarchical follicles and egg production rate. Johnson et al. (1993) reported that circulating concentrations of FSH increased after removal of large follicles (the primary source of inhibin, which negatively feeds back an FSH but not LH secretion). A rise in FSH concentrations (above 2 ng/ml) was found a few weeks before initiation of egg production, and a decline (below 2 ng/ml) was found
after starting laying in chickens (Vanmontfort et al., 1995; Johnson and Brooks, 1996; Lewis et al., 1999; Lovell et al., 2001). Relatively stable FSH concentrations of below 2 ng/ml in plasma have been reported during the ovulatory cycle in chickens, whereas preovulatory surges of LH and P4 occur (Scanes et al., 1977; Vanmontfort et al., 1994; Lovell et al., 2000). Plasma FSH concentrations increased above 2 ng/ml when the laying chicken hens were subjected to induced cessation of egg laying by short term food deprivation (Vanmontfort et al., 1994; Lovell et al., 2000).

**Historical background information of leptin:**

Leptin expression in adipose tissue indicates the status of fat stores to the hypothalamus and plays an important role in energy homeostasis (Houseknecht et al., 1998; Ahima and Flier, 2000, Friedman, 2002, Sahu, 2004a, b). Leptin was originally identified as the product of the ob gene in the mouse and is a protein with 146 amino acids and 16 kDa molecule weight (Zhang et al., 1994). It is secreted into the bloodstream and acts on hypothalamic leptin receptors to control appetite. Two obese mutant mice were identified over 40 years ago. It has been shown that two distinct single genes were mutuated on chromosome 6 (ob) and 4 (db) (Ingalls et al., 1950; Hummel et al.,
The gene encoding mutated leptin and defective leptin receptor were discovered in \textit{ob/ob} and \textit{db/db} mice, respectively, resulting in genetic obesity syndromes (Zhang et al., 1994; Campfield et al., 1995; Pelleymounter et al., 1995; Stephens et al., 1995). \textit{Fa/fa} rats also have a dysfunctional leptin receptor (Phillips et al., 1996; White et al., 1997). Both \textit{db/db} and \textit{fa/fa} rats with dysfunctional leptin receptors have abnormally large fat reserves, not caused by leptin itself. Leptin is mainly secreted from adipocytes and plays an important role on the hypothalamus to regulate feed intake, body energy stores, and has effects on reproduction.

Leptin is also secreted by bone marrow adipocytes (Laharrague et al., 1998), cells in the epithelium of the stomach, in the placenta (Hoggard et al., 1997; Senaris et al., 1997; Bado et al., 1998), and avian liver (Taouis et al., 1998; Friedman-Einat et al., 1999). Leptin mRNA has been identified in human placenta and stomach (Senaris et al., 1997; Bado et al., 1998) and chicken embryonic liver and yolk sac (Ashwell et al., 1999). Studies with obese and non-obese animals have shown that a strong positive correlation between circulating leptin concentrations and percentage of body fat (Hamilton et al., 1995; Maffei et al., 1995; Klein et al., 1996). Anatomic findings indicate that the arcuate nucleus of the hypothalamus (ARH), which regulates energy homeostasis, are
structurally and functionally immature until the third week of postnatal life in mice (Bouret and Simerly, 2004). During 2 to 3 postnatal wk exogenous leptin did not inhibit growth, food intake, or energy expenditure (Mistry et al., 1999; Ahima and Hileman, 2000; Proulx et al., 2002)

**Leptin gene:**

The gene encoding leptin has been cloned in chicken (Taouis et al., 1998; Ashwell et al., 1999), human and mice (Zhang et al., 1994), rat (Murakami and Shima, 1995), ovine (Dyer et al., 1997b), bovine (Ji et al., 1998), procine (Bidwell et al., 1997), and monkey (Hotta et al., 1996). Chicken leptin has only 145 amino acids rather than 146 amino acids in mammalian sequence (Taouis et al., 1998; Ashwell et al., 1999). Chicken leptin, in contrast to mammalian species, contains an unpaired cystine at position 3 of original cDNA. In avian species, the leptin gene is expressed in adipose tissue, embryonic liver, and yolk sac (Ashwell et al., 1999).

At least two laboratories confirmed the presence of a leptin homolog in chicken and verified its sequence (Taouis et al., 1998; Ashwell et al., 1999). A first study of cloning and the sequence of chicken leptin gene was reported by Taouis et al. (1998) that
presence of a leptin homolog had a unique expression liver and adipose tissue. The chicken leptin sequence was obtained by Reverse Transcription Polymerase Chain Reaction (RT-PCR) from chicken fat and liver mRNA. The primers are based on the mouse leptin sequence (Taouis et al., 1998). Chicken leptin cDNA (Genebank AF 012727, AF082500) is >90 identical to murine leptin and >80% identical to other known leptin sequences (Taouis et al., 1998, 2001; Ashwell et al., 1999). Turkey leptin cDNA has been cloned as well. The sequence was nearly identical to the chicken leptin sequence (AF082501). A controversy over whether the chicken leptin gene has been cloned was addressed by Friedman-Einat et al. (1999). They failed to amplify a mouse-like leptin sequence from chicken RNA or genomic DNA using primers that completely matched those of the published mouse and chicken sequences (Friedman-Einat et al., 1999). Friedman-Einat et al. (1999) pointed out that since the sheep and mouse leptin nucleotide sequences are 83% homologous, it is improbable that putative chicken leptin sequences should be more than 95% homologous with the mouse sequence. In addition, the chicken leptin genomic sequence is still unknown.
**Leptin receptor:**

The leptin receptor is a member of the cytokine receptor family (Tartaglia et al., 1995, Tartaglia, 1997). Hypothalamic leptin binding and gene expression of leptin receptor were up regulated by fasting, suggesting low leptin concentrations during fasting were involved in modulation of leptin receptor expression (Lin and Huang, 1997; Baskin et al., 1998, 1999; Sahu et al., 2002). In chicken, the leptin receptor and the expression of leptin receptor have been demonstrated in central (hypothalamus) and peripheral tissues (Horev et al., 2000; Ohkubo et al., 2000; Benomar et al., 2003). The leptin receptor gene has also been mapped to chicken chromosome 8 (Dunn et al., 2000). Molecular cloning and gene expression of the leptin receptor were reported in turkey (Richards and Poch, 2003), chicken (Horev et al., 2000; Ohkubo et al., 2000), ewe (Dyer et al., 1997a), bovine (Chelikani et al., 2003), and pig (Lin et al., 2001). Leptin receptor gene expression was down regulated by recombinant chicken leptin in hepatoma cells of male chickens whereas leptin desensitizes it own response, which ligand-induced leptin mechanism may be involved, was also reported in mammals (Barr et al., 1999; Uotani et al., 1999; Tena-Sempere et al., 2000; Cassy et al., 2003). Massive obesity in humans
and rodents was associated with a deficiency in leptin action due to mutation in leptin receptor or leptin gene (Friedman and Halaas, 1998; O’Rahilly et al., 2003).

**Effect of administration of leptin on physiological responses:**

Direct evidence has shown in rats and humans that daily administration of recombinant leptin resulted in reducing food intake within a few days in *ob/ob* and lean wild type mice but decreasing food intake was not found in *db/db* mice (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995; Brunner et al., 1997; Flynn and Plata-Salaman, 1999). At least two fundamental effects could explain body weight changes when animals were administrated leptin (Pelleymounter et al., 1995). First is reduced feed consumption, mediated by inhibition of neuropeptide Y synthesis since neuropeptide Y is a very potent stimulator of feeding behavior (stimulator of appetite). It has been reported that hypothalamic neuropeptide Y is a potential target for leptin action via the co-localization of leptin receptor mRNA with neuropeptide Y gene expression (Cunningham et al., 1999). Leptin inhibited the synthesis of neuropeptide Y in the arcuate nucleus of the hypothalamus (Cusin et al., 1996; Erickson et al., 1996b). Direct evidence has shown that the gene expression of neuropeptide Y was decreased
after an administration of leptin in rodents (Stephens et al., 1995; Ahima et al., 1996) and was increased after 24 h or 48 h fasting in hypothalamus of quail, layer and broiler chickens (Boswell et al., 1999a, b, 2002).

Studies in mammals suggested that GnRH and LH secretion could be regulated by neuropeptide Y (Rodent; Kalra, 1993, Primate; Kaynard et al., 1990). The LH secretion was inhibited by the central administration of neuropeptide Y in pig and inhibition of food intake by leptin was reversed after treatment with neuropeptide Y (Barb, 1999). Chicken neuropeptide Y receptor has been identified and characterized at the gene and protein levels (Holmberg et al., 2002). Studies from hypothalamic tissue in vitro demonstrated that acute release of neuropeptide Y was not affected by a leptin treatment (Jang et al., 2000; King et al., 2000). Indirect evidence suggested that activation of neuropeptide Y system was a chronic physiological change during fasting and chronic leptin administration (Stephens et al., 1995; Schwartz et al., 1996b; Schwartz et al., 1998). Neuropeptide Y knock-out mice showed normal feed intake and energy balance phenotypes (Palmiter et al., 1998). Erickson et al. (1996b) reported that leptin is independent from neuropeptide Y because mice deficient for neuropeptide Y have normal food intake and body weight. In addition, the mice which lack of neuropeptide
Y were more sensitive to leptin (Erickson et al., 1996a), suggesting leptin acts through other pathways to maintain normal food intake and body weight with the absence of neuropeptide Y in hypothalamus. Mutant mice decreased food intake and lost weight in comparison to control mice when treated with recombinant leptin, suggesting neuropeptide Y is not essential for certain feeding responses or leptin actions but is an important modulator of appetite. Secondly mutant mice increased energy expenditure indicated by increased oxygen consumption, higher body temperature, and loss of adipose tissue mass (Woods et al., 1998; McMinn et al., 2000; Jensen, 2001; Berthoud, 2002; Blevins et al., 2002).

**Relationships between leptin and feeding treatments:**

In the human and rat, leptin secretion and expression increased during refeeding or overfeeding (Kolaczynski et al., 1996; Sinha and Caro, 1998; Marzolo et al., 2000) and decreased during overnight fasting (Boden et al., 1996; Kolaczynski et al., 1996; Vuagnat et al., 1998) and by reduced body weight (Maffei et al., 1995; Considine et al., 1996). A body weight decrease of about 10% in obese women resulted in a decrease of about 50%
in leptin concentration whereas a body weight increase of about 10% resulted in an about 3 fold increase of leptin concentrations (Considine et al., 1996; Kolaczynski et al., 1996).

Leptin may be up regulated by repeated cycles of fasting and refeeding (Kim and Scarpace, 2003). In this study, male rats were treated with repeated cycles of 1-d fasting and 1-d refeeding for 42 d. The repeated fasting and refeeding rats gained less weight in comparison to control rats and daily food intake was reduced after d 16. The treated male rats also had higher circulating fasting leptin concentrations at d 35 and the end of experiment in comparison to control rats (Kim and Scarpace, 2003). Different results were reported by Ernsberger et al. (1996) where repeated cycling resulted in a greater body weigh gain in the obese spontaneous hypertensive rat of a model of genetic obesity. These two studies suggest that the genetic background may affect the response to weight cycling. It also suggested that body weight might be controlled primarily by food intake instead of meal timing. Young laying chicken hens (34 wk of age) injected with recombinant chicken leptin (250 ug kg\(^{-1}\)) twice a day have shown delayed cessation of egg laying, attenuated regression of yellow hierarchical follicles, altered ovarian steroidogenesis, and abolished the fasting-induced apoptosis during fasting (Paczoska-Eliasiewicz et al., 2003).
Effect of leptin injection on food intake, energy metabolism, and body weight:

It has been shown after leptin treatment there was a dose dependent decrease in food intake, fat depots, and body weight, but increased energy metabolism (Pelleymounter et al., 1995; Levin et al., 1996). A number of studies in mammals have reported that intracerebroventricular or intrahypothalamic injections of leptin decreased food intake (Swine: Barb et al., 1998; Mice: Chen et al., 1996; Rat: Cusin et al., 1996). A recent study has shown in great tits (wild bird) that a single intramuscular injection of recombinant chicken leptin (10 ug) after overnight fasting decreased food intake (Lohmus et al., 2003). Similar results also were found in domestic chickens that food intake decreased after intracerebroventricular (Denbow et al., 2000) and intravenous injections of leptin (Dridi et al., 2000).

Food intake was inhibited in young (2-d old) and older (5-wk old) male layer chickens after a single intraperitoneal (ip) injection of ovine or chicken leptin (1 mg/kg, Dridi et al., 2000). This study showed that the total cumulative food intake and time spent on eating were reduced but the number of approaches to the feeders was not changed in
the chickens with ovine or chicken leptin treatments in comparison to control chickens (Dridi et al., 2000).

Recombinant mouse leptin (0.1 or 1 ug) was injected into albumen of eggs on day 5 of during embryonic development of Japanese quail, and the quails were hatched earlier (5 to 24 h) and had higher body weight and expressed a higher growth rate in comparison to the control group, suggesting leptin acts as a signal of low energy status to improve utilization of nutrients (Lamosova et al., 2003). Food intake was not affected when adult male Japanese quails (8 wk of age) were injected daily intramuscular with murine recombinant leptin for 4 d (Macajova et al., 2003) but an inhibitory effect of leptin and mutated analog (Cys 3) on food intake was found in young chickens (Dridi et al., 2000).

Denbow et al. (2000) reported in layer and broiler chickens that food intake was reduced after intracerebroventricular administrarion of human leptin (10 ug). A study in rodents with leptin treatment has shown a slightly different result in that food intake (meal size) was reduced but meal duration and meal frequencies were not changed (Flynn and Plata-Salaman, 1999). A study reported in male chicken that intracerebroventricular administration of mouse leptin (<5.0 ug), which showed 97%
homology to chicken leptin, did not reduce food intake, suggesting either mouse leptin does not efficiently bind to chicken leptin receptor or the leptin receptor may be absent in the chicken brain (Bungo et al., 1999).

**Rhythm of peripheral leptin concentrations:**

Leptin concentrations increased during puberty (Mice: Chehab et al., 1997; Pig: Qian et al., 1999) but the increase was not found before onset of puberty in a primate (Plant and Durrant, 1997; Plant, 2001a, b, 2002). Blood leptin concentrations are diurnally regulated peaking at night in human and rodents (Saladin et al., 1995; Sinha et al., 1996). The diurnal rhythm of plasma leptin concentrations was entrained to meal times rather than a circadian oscillator in humans (Sinha et al., 1996; Schoeller et al., 1997; Langendonk et al., 1998). There is no evidence, however, to show that a diurnal rhythm of plasma leptin concentrations was independent from either meal timing or photoperiod in sheep (Blache et al., 2000; Marie et al., 2001). High leptin secretion found at night was associated with night time feeding in rodents (Saladin et al., 1995; Ahren, 2000).
Horton et al., (2000) reported in seasonal Siberian hamsters no nocturnal increase in leptin in either long-day or short-day photoperiods. A study has shown in women sampled every 7 minutes for 24 hr that leptin concentrations diurnally fluctuated and rose nocturnally (Licinio et al., 1998a). The synchronicity of LH and leptin occurred late at night. Fluctuations in leptin expression were also reported in Diugarian hamsters, with leptin gene expression reduced during winter or during exposure short photoschedule but the change was independent in environmental temperature (Klingenspor et al., 1996). Studies have shown in rodents that a peak in leptin concentration was associated with the initiation of eating behavior, and the increase was inhibited by fasting, suggesting leptin may be involved in daily food intake and short term regulation of body weight (Frederich et al., 1995; MacDougald et al., 1995; Xu et al., 1999). A study showed in human that nocturnal increase in leptin concentration is independent from feeding treatment (Sinha et al., 1996). It suggested two different leptin regulations might occur in rodent and human.
Leptin resistance:

In most cases, obese humans are hyperleptinemic, suggesting they are leptin resistant (Lonnqvist et al., 1995; Hassink et al., 1996; Schwartz et al., 1996a). The potential mechanism of leptin resistance may mediate impairment of brain leptin transport, abnormality of leptin receptor, or leptin postreceptor signaling (Sahu, 2004a). The obese mice and humans had very high circulating leptin concentrations but maintained normal food intake (Maffei et al., 1995; Halaas et al., 1997). A rat model of chronic central leptin infusion (160 ng/h) for 28 d demonstrated that initial increase in food intake was induced and then recovered to a normal level by 2 wk of infusion (Sahu et al., 2002). In these rats, the food intake remained normal throughout the rest of 4 wk of leptin infusion, suggesting the rats developed leptin resistance to the satiety behavior. Body weight was gradually decreased to a nadir by 12 d of leptin infusion and then it remained stable at a low level even though the rat consumed similar amount of food in comparison to control rats, suggesting the development of leptin resistance was induced in the hypothalamic neuropeptide Y neurons because the gene expression of neuropeptide Y decreased during early stage of leptin infusion and were not different from control rats at the middle stage of infusion (d 15) (Sahu et al., 2002; Sahu, 2004a).
Leptin and reproduction:

It is well accepted that an amount of body fat is one of the important factors controlling the onset of puberty and maintenance of adult reproduction. Beltranena et al. (1993) reported that a decrease in the reproductive function was nutritionally induced that occurs without a change in body fat. It is thought that leptin plays important roles in puberty development and maintenance of female reproductive function. A study of cultured pituitary cells from 4, 6, and 8 wk of age female rats with leptin and GnRH showed that LH and FSH secretion could be induced by leptin with presence or absence of GnRH (Tezuka et al., 2002). Several studies reported the effect of leptin on GnRH and LH secretion (Ahima et al., 2000; McCann et al., 2001; Steiner et al., 2003; Barb et al., 2004).

Pulsatile leptin secretion is positively correlated with gonadotropins and E₂ in normal cycling women (Licinio et al., 1998a, b). Secretion of GnRH was stimulated by leptin in vivo in rat (Watanobe, 2002) but a recent study has shown in prepubertal monkeys that inducing secretion of GnRH failed after continuous peripheral infusion of leptin (Barker-Gibb et al., 2002). Administration of leptin reversed the effect of fasting
on pulsatile secretion of LH, increasing plasma concentrations of LH and FSH in fasted mice and ob/ob mice (Ahima et al., 1996; Barash et al., 1996; Nagatani et al., 1998). Similar results also were seen in the study of prepubertal heifers that exogenous leptin can prevent the fasting-mediated reduction in frequency of LH pulses and modified the release of LH stimulated by GnRH (Maciel et al., 2004).

After acute injection of estrogen primed ovariectomized rats, leptin treatment stimulated LH and FSH secretion from cultured pituitaries (Yu et al., 1997c, d). Releasing gonadotropin releasing hormone from hypothalamic explants was stimulated by leptin (Yu et al., 1997c). These results suggested that the regulation of reproductive hypothalamic-pituitary-gonadal axis was modulated by a central action of leptin. Carro et al. (1997) reported that LH secretion was prevented by intracerebroventricular injection of leptin antiserum which supported other studies. Studies also reported that sexual maturation was advanced and uterine and ovarian weight was increased when restricted-fed and ad libitum-fed animals were treated with leptin (Ahima et al., 1997; Cheung et al., 1997; Chehab et al., 1997).

A recent study in gilts has shown that serum LH concentration was not affected when prepuberal gilts received intracerebroventricular (i.c.v.) leptin injection, suggesting
the inability of i.c.v. leptin injection to increase LH secretion may be related to stage of sexual maturation (Barb et al., 2004). That study also demonstrated in maintenance-fed prepuberal gilts (180 d of age) that basal LH secretion in anterior pituitary cells was directly stimulated by leptin treatment but the stimulation was inhibited by combination of GnRH and leptin (Barb et al., 2004). Similar results were found in rats at 6 wk of age that an increase in basal LH concentration was induced by leptin treatment in a dose-dependent manner, but GnRH-induced LH secretion was not stimulated in anterior pituitary cell cultures, suggesting leptin modulated anterior pituitary function and LH secretion (Ogura et al., 2001).

Plasma LH concentrations decreased in food-restricted sheep (Foster et al., 1985; Henry et al., 2004) and restricted-fed broiler breeder hens (Burggeman et al., 1999). The importance of leptin under different nutritional status (fasting or ad libitum) in regulation of LH secretion was studied in ovariectomized ewes (Henry et al., 2004). The data showed in ovariectomized ewe after infusion of leptin (50 ul/h for 72 h) into the third cerebral ventricle that plasma LH secretion was similar to control ewes but LH pulse amplitude was higher in the fasted-treated (for 72 h) ewes (Henry et al., 2004).
Peripheral concentrations of insulin and glucose and feeding treatment:

The chicken insulin receptor substrate-1 gene (IRS-1) has been cloned (Taouis et al., 1996) to study insulin signal in the chicken (Dupont et al., 1999). Genetically fat and lean chickens were obtained by divergent selection for abdominal fat pad weight in male at 9 wk of age (Leclercq et al., 1980). Leclercq et al. (1980) reported that fat chickens exhibited higher plasma insulin concentrations which can not be explained by insulin resistance as in obese mammals (Heydrick et al., 1993). After overnight starvation, genetically fat chicken are more sensitive to exogenous insulin (Saadoun et al., 1988). The differences in glucose tolerance were found in chicken lines selected for high or low abdominal fat weight but the differences were not found in plasma insulin concentrations, suggesting chicken with high abdominal fat weight was different from obese mammals because the chicken did not exhibit hyperinsulinemia or insulin resistance (Touchburn et al., 1981). However, Burkhart et al., (1983) reported that electrolytic lesions of ventromedial hypothalamus failed to exhibit hyperphagia or gaining body weight in heavy body weight chickens because mature heavy body weight chickens might be already hyperinsulinemia. Obesity syndrome was induced by lesions of ventromedial hypothalamus in chickens with light body weight.
Insulin is involved in glucose metabolism in birds as well as in mammals (Harvey et al., 1978; Leclercq et al., 1988; Richards, 2003). In contrast to most mammals, blood glucose concentrations are about twice as high in chickens. In contrast to mammals (Gilt: Barb et al., 1997, 2001; Human: Grinspoon et al., 1997; Smith et al., 1995; Rat: Emler and Schalch, 1987; Ewe: Morrison et al., 2001), only a minor change in blood glucose concentration was found following short term starvation by maintaining a high blood glucose level during prolonged fasting (Hazelwood and Lorenz, 1959; Belo et al., 1976). Decreased blood concentrations of glucose and LH pulses induced by administration of insulin have been reported in rats (Goubillon and Thalabard, 1996; Cagampang et al., 1997), sheep (Clarke et al., 1990; Medina et al., 1998), goats (Ohkura et al., 2004), and monkeys (Heisler et al., 1993), suggesting insulin may play a role as a metabolic signal to modify the pulsatile release of GnRH from the hypothalamus.

Glucose transport across cell membrane is mediated by glucose transporter proteins (GLUT). Four isoforms (GLUT-1, 2, 3) of the GLUT family have been identified in birds (White et al., 1991; Wang et al., 1994; Wagstaff et al., 1995). The GLUT4 isoform is dominantly expressed in muscle and adipose tissue and plays an important role in insulin-responsive glucose transport in mammals (Watson and Pessin,
The GLUT4 protein was undetectable in chicken embryonic skeletal muscle (Carver et al., 2001). The GLUT8 isoform has been reported to be an insulin-responsive glucose transport in the blastocyst and skeletal muscle in rat (Carayannopoulos et al., 2000; Lisinski et al., 2001). A study of expression of GLUT transporters in the broiler chickens suggests that the GLUT8 isoform is expressed in chicken brain, kidney, and spleen but the GLUT4 isoform is deficient in chickens (Seki et al., 2003). This suggests a deficiency of the GLUT4 transporter in chickens may explain why chickens are hyperglycemic and insulin resistant.

In contrast to mammals, chickens are highly resistant to administration of high dosages of insulin (Hazelwood and Lorenz, 1959; Simon, 1989; Akiba et al., 1999; Chida et al., 2000) or diabetogenic drugs (Simon and Dubois, 1980; Danby et al., 1982; Simon, 1989). Borron et al. (1979) reported that lipoprotein lipase activity was stimulated by very high concentrations of insulin in adipose tissue. Disorganization of insulin secretion is a feature of animal models of obesity (Beales and Kopelman, 1996). It has been reported that increased insulin release in response to a meal or a glucose load was associated with genetic and hypothalamic obesity in mammals (Steffens, 1970; Stern et al., 1972; Bryce et al., 1977; Inoue et al., 1977).
Peripheral insulin is secreted in a pulsatile fashion in young turkeys (Anthony et al., 1990). The pulsatile secretion was eliminated in fasted birds. The half-life of insulin was reported to be 7.5 and 8.7 min between laying and non-laying turkey hens, respectively (McMurtry et al., 1987). The concentration of insulin decreased after overnight fasting in comparison to ad libitum-fed chickens (Simon and Rosselin, 1978) and turkeys (Anthony et al., 1990). The decreased concentration of insulin was abolished and insulin level was rapidly elevated after refeeding following 24 h of fasting (Anthony et al., 1990) or after an oral administration of glucose (Simon and Rosselin, 1978).

Glucose concentrations (220 to 250 mg/dL) were relatively constant in fed and fasted young turkeys (Anthony et al., 1990; Kurima et al., 1994b). The concentration of triacylglyceride (TG) was lower and stable in the feed deprived turkeys than in fed turkeys (0.33 mg/mL and 0.71 mg/mL, respectively). Glucose infusion stimulates leptin in fasting human. The study in turkey has shown that concentrations of growth hormone (GH) decreased after glucagon infusion in ad libitum and feed deprived young turkey hens (Kurima et al., 1994a). This study also has shown that concentrations of both glucose and nonesterified fatty acids (NEFA) dose-dependently increased after
glucagon infusion, suggesting glucagon is very important to mobilize carbohydrate and lipid metabolism in growing female turkeys.

Plasma glucose concentration was elevated by a single administration of glucagon in laying chicken hens (Mitchell and Raza, 1986) and turkey hens (McMurtry et al., 1996). A greater increase of glucose was found in ad libitum turkeys in comparison to feed deprived turkeys (Kurima et al., 1994a). The plasma glucose concentration was lower in a selected fat line of chickens than in a selected lean line of chickens, both in the fed and fasted states after hatching (Simon and Leclercq, 1982). In chickens, fattening is not associated with insulin resistance. A study has shown that genetically fat chickens are more sensitive to exogenous insulin in a feed deprived state (Saadoun and Leclercq, 1987). Hammouda et al. (1992) also reported that turkeys with feed deprivation are much more sensitive to insulin infusion than ad libitum fed turkeys.

Continuous infusion of bovine insulin at 22.5U/kg BW/d induced persistent hypoglycaemia (about 50% of normal blood glucose concentrations) lasting for 4 d in 4 wk of age broiler chickens (Akiba et al., 1999). Chronic hypoglycemia lasting for more than 5 d induced by administration of insulin was reported in the rat (McCormick et al., 1978) and the human (Caprio et al., 1989). Foltzer et al. (1981) reported that inhibition
of growth hormone secretion was induced by intravenous infusion of insulin (2.5 or 10 
mu/kg per min for 30 min) and glucagon (0.1 or 0.5 ug/kg per min for 30 min) in growing 
ducks (4 to 6 wk of age), suggesting insulin and glucagon have direct effects on the 
secretion of growth hormone. Increased plasma concentration of NEFA and decreased 
plasma concentrations of TG and insulin induced by overnight fasting was reported in 
turkey (Bacon, 1986; Anthony et al., 1990), geese (Nir et al., 1973; March, 1984).

It has been shown that peripheral concentrations of leptin correlated closed to 
body weight and body adiposity in mice (Ahren et al., 1997). Leptin concentrations 
were elevated after exogenous administration of insulin (Kim et al., 1998; Mueller et al., 
1998; Wang et al., 1998). Studies have shown that diurnal variation in peripheral 
concentrations of leptin is dependent on gender (higher in female than in male), food 
intake and circulating insulin in mice (Ahren, 2000) and in humans (Licinio et al., 1998a; 
Havel et al., 1999). It is suggested that diurnal pattern of leptin may be regulated by the 
level of insulin and glucose. The nocturnal rise in leptin level correlates to diurnal 
variation in circulating insulin in response to meals but the increase was abolished by 
fasting (Saad et al., 1998).
Triglyceridemia was higher in the fat line (selection for high level of abdominal fat) than in the lean line (selection for low level of abdominal fat) of chickens (Leclercq et al., 1984). This suggested that the secretion of VLDL TG was increased by the higher triglyceridemia of the fat line of chickens. Plasma glucose-insulin relationships were studied in two lines of adult cockerels selected for high and low residual feed consumption. For a given body weight, the high residual food consumption birds had a 74% higher food intake than the low residual food consumption birds (Gabarrou et al., 2000). Plasma glucose concentrations were not different between the lines with ad libitum-feeding but the concentrations declined after feed deprivation in both lines (Gabarrou et al., 2000). Plasma insulin concentrations were lower in high residual food resumption cockerels in comparison to low residual food consumption cockerels when either ad libitum-fed or fasted.

There was a positive relationship between growth rate and plasma insulin-like growth factor-1 (IGF-I) concentrations in growing chickens (Scanes et al., 1989; McGuinnes and Cogburn, 1990). Circulating IGF-I concentrations increased greatly with age until chickens with either a high (6 wk of age) or a low (12 wk of age) growth rate reached a body weight of about 1 kg (Beccavin et al., 2001), then declined in older or
heavier birds (Johnson et al., 1990; McGuinness and Cogburn, 1990; McMurtry et al., 1994; Radecki et al., 1997). It has been postulated that IGF-I may be associated with the energy regulating system in both growth and reproductive process. Studies have shown that IGF-I stimulated GnRH release from the hypothalamus in vitro in the rat (Hiney et al., 1991) and enhanced LH secretion from the anterior pituitary in the pig (Whitley et al., 1995).

**Follicular development and postovulatory follicles associated with unreconciled ovulations:**

The technique of feeding or injecting fat-soluble dyes has been used to monitor the period of rapid yolky follicular development in turkey (Bacon and Cherms, 1968), quail (Bacon and Koontz, 1971), domestic pigeon (Birrenkott et al., 1988), and broiler chicken hens (Yu et al., 1992a). The feeding of different colored fat-soluble dyes on alternate days showed that ovarian yolky follicles of ad libitum-fed hens had a longer period of development than restricted-fed hens after recruitment into the rapid stage of development and entrance into the hierarchy (Yu et al., 1992a). Williams and Sharp
(1978) suggested that lower egg production of ad libitum-fed hens might be due to internal laying, follicular atresia, or the oviposition of shell-less eggs.

Bacon and Cherms (1968) reported that in turkey hens the initiation of rapid yolk deposition was between 4 and 11 d after photostimulation, and that a relatively small number of yellow yolky follicles began yolk deposition and entered into the hierarchy at that time. In a study over a 3-d period, in a dam line of turkeys rapid yolk deposition may continue for 18 and 21 d after photostimulation (Melnychuk et al., 1997). Birrenkott et al (1988) reported that the rapid follicular development was 8.3 d in guinea fowl and 6.4 d in pigeons. The duration of the period of rapid development was similar in ad libitum and restricted-fed turkey hens and averaged 11.6 d when both groups were switched to ad libitum feed at 30 wk of age (Hocking et al., 1987b).

Unreconciled ovulations were defined by Renema et al. (1995) as the number of postovulatory follicles that can not be accounted for by previous oviposition and eggs, or eggs in the oviduct. Renema et al. (1995) reported in sire line turkey hens that the average unreconciled postovulatory follicles were 4.9. They suggested that the presence of unreconciled postovulatory follicles might be due to internal ovulations. Melnychuk et al. (1997) reported that the number of unreconciled postovulatory follicles was greater
in sire line turkeys than dam line turkeys (3.0 vs 1.6, respectively). First ovipositions occur when the ovary and oviduct are developmentally mature. Internal ovulations might occur when the ovary matured earlier than the oviduct and the immature infundibulum might not efficiently sequester the ovulated ovum to form a hard shelled egg. The oviduct of both sire and dam lines and the ovary of the sire line reached their mature weight at 26 d after photostimulation, whereas the ovary of the dam line reached the mature weight 3 d later (Melnychuk et al., 1997). They suggested that fewer unreconciled postovulatory follicles in dam line turkeys might be due to full development of the oviduct earlier than ovary, and that the ovulated ovum may be more successfully sequestered by the mature oviduct.

**Number of yolky follicles and egg production:**

Ovarian morphology of ad libitum-fed broiler hens was compromised in comparison to restricted-fed hens. The ad libitum-fed hens lost the capability to regulate the recruitment of follicles into the hierarchy, resulting in more and multiple hierarchical follicles in the ovary. Increasing body weight was induced by ad libitum feeding of hens switched from restricted feeding. Robinson and Wilson (1996) reported
that after returning restricted-fed broiler hens to ad libitum-feeding, body weight increased by 500 g after 7 days, and 690 g after 14 days. A similar result in broiler breeder hens was reported by Robinson et al. (1991) that body weight was heavier by approximately 700 g in ad libitum-fed than restricted fed hens throughout the laying period. The number of ovarian follicles was significantly increased from 5.3 to 7.0 after ad libitum feeding. These observations suggest that loss of regulation of the ovarian follicular hierarchy can occur in a short period of time of ad libitum-feeding, but the greater number of hierarchical follicles does not contribute to higher egg production with ad libitum feeding. The increased number of follicles resulted in multiple ovarian hierarchies and higher incidence of multiple-yolked eggs being oviposited by broiler breeder females (Yu et al., 1992a).

A female line of turkeys had a greater proportion of follicles in single and double hierarchical arrangement than a male line, whereas the male line turkey had a greater proportion of follicles in triple or quadruple (or larger) hierarchical arrangement than the female line (Melnychuk et al., 1997). In broiler breeder hens, the incidence of multiple ovulations was decreased by feed restriction to control body weight gain during the period before sexual maturity (Hocking et al., 1989). Hocking (1993) concluded
that the incidence of multiple ovulations can be decreased by 14 wk of restricted feeding of broiler breeders, resulting in improved egg production. A positive association between body weight at sexual maturity and the number of developing yolky follicles has been reported (Hocking, 1990, 1992, 1993). The number of yellow yolky follicles in broiler breeders increased following ad libitum feeding of restricted fed hens after photostimulation at the onset of egg production (Hocking, 1996). The ovary weight was heavier in ad libitum-fed broiler breeder hens during both rearing and breeding periods but no difference in oviduct weight was observed at sexual maturity (19 to 28 wk of ages; Yu et al., 1992a). In summary, more yolky follicles in ad libitum-fed broiler breeder hens are associated with a lower egg production rate.

Effects of feeding regimes, hen age, and follicle stimulating hormone (FSH) on follicular growth:

Yu et al. (1992a) observed that the number of yolky follicles in ad libitum-fed broiler breeder hens declined from 12.2 at sexual maturity (19 to 28 wk of age) to 6.9 at 62 wk of age. Similar observations for restricted-fed broiler hens were 7.8 at sexual maturity and 4.6 at 62 wk of age. These declines in the number of yolky follicles were
coincident with the normal decline in egg production rate with aging. Restricted feeding
broiler breeder hens decreased the number and atretic yolky follicles and the proportion
of multiple ovulations (Hocking and Robertson, 2000). Also, restricted feeding
decreased the number of small white follicles (1.4 to 2.4 mm) and increased the number
of large white follicles (2.4 to 5.0 mm in diameter). In addition, the weight of the
largest follicle was greater in restricted fed hens compared to ad libitum fed hens.
Palmer and Bahr (1992) suggested that an inefficiency of follicle production might be a
cause of reduced egg production. The average number of large follicles was increased
from 5.8 to 7.0 after 14 d of ad libitum feeding after switching hens (at 44 wk of age)
from restricted feeding (Robinson et al., 1993).

Increased feed allocation did not stimulate increased ovarian development or
increased egg production in 54-wk-old broiler breeder hens (McGovern et al., 1997).
Their data suggested that follicle development in older broiler breeders is less sensitive to
over-feeding than in young hens. The study has shown that exogenous treatment with
FSH stimulated follicular recruitment into the hierarchy (Palmer and Bahr, 1992).
However, no FSH concentrations were measured between control and over-feed
treatments related to follicular development in that study. Bruggeman et al (1999)
reported that plasma concentrations of FSH were not different between restricted-fed and ad libitum-fed broiler breeder hens when they were at 15 wk of age and age at first egg, but were increased when they were at 18 wk of age. No further evidence was found indicating that higher plasma FSH levels in ad libitum-fed broiler breeder hens are associated with a greater number of yolky follicles in comparison to a lesser number of yolky follicles in restricted-fed hens. The decline in egg production may be related to a decrease of FSH secretion, but egg production rate may not be related to FSH early in reproduction. New evidence, including changes in hourly FSH concentrations in plasma during ovulation and oviposition cycles should be obtained to clarify the relationship between follicle number and FSH.

**Body weight and age at onset of sexual maturity:**

The relationship between age and body weight is complex at sexual maturity (Bornstein et al., 1984; Soller et al., 1984). Turkey hens reached their maximal body weight shortly after photostimulation, before the onset of sexual maturity (approximately 33 wk of age), and then gradually declined after initiation of egg production (Bacon and Nestor, 1982; Lilburn and Nestor, 1993; Applegate and Lilburn, 1996). The loss of
body weight during the egg production period was associated with a relative decrease of abdominal fat (Applegate and Lilburn, 1996). A low energy diet resulted in decreasing body weight of broiler breeder hens with increasing age at the onset of egg production (Pearson and Herron, 1982). Bartov et al. (1994) reported that average body weight of broiler breeder hens was similar at first eggs between hens fed ad libitum on low-protein diets, but the average age at first egg was different. It was suggested that the onset of egg production, after reaching a critical age, was dependent on body weight (Bornstein et al., 1984; Soller et al., 1984; Yu et al., 1992b). The effect of age and body weight on the response to photostimulation (PS) was studied in turkey hens (Applegate et al., 1997). The turkey hens were separated into 2 body weight groups (1-kg difference) at 24 to 25, 27 to 28, and 31 to 32 wk of age and LH secretion measured before and 3 d after photostimulation. The results showed that hen age affected the response to photostimulation, but not the relatively small difference in hen body weight. A change in LH baseline concentrations was greatest in hens at 24 to 25 wk of age, suggesting photostimulatory response (PR) is more active at an early age in turkey hens. Increased body weight during rearing advanced the age at first egg in broiler breeders (Blair et al., 1976; Robinson et al., 1986; Yu et al., 1992a).
Photostimulation and egg production:

Egg production begins about 2 to 3 wk after photostimulation in turkeys (Chapman et al., 1994; Liu et al., 2001a, c). A model for persistency of egg production proposed by Grossman et al (2000) is that peak egg production is maintained for several weeks and then gradually declines. The pattern of egg production in restricted-fed broiler breeder hens was characterized by a sharp increase up to 90% (peak egg production) after photostimulation with a shorter duration of peak production followed by a decline with advancing age of laying hens to 50% at 40 wks of production (Arbor Acres, 2000). Continuous lighting (24L:0D) has been used to stimulate egg production in comparison to day-night cycle (i.e., 15.25L:8.75D). Early egg production of turkey hens (Egg line) was not different between 14L:10D and continuous lighting (Bacon et al., unpublished data). Gow et al (1987) reported that broiler breeder hens had similar egg production under photostimulation of both lighting conditions but the mean interval between ovipositions within a sequence was significantly longer with continuous lighting in comparison to day-night cycle lighting (26.4 and 25.3 h, respectively). Selected White Leghorns had similar egg production (86/100 d⁻¹) under both lighting treatments.
whereas selected Australorps had more eggs (95.6±2.0 /100 d⁻¹) in continuous lighting in comparison to Long Day lighting (90.3±1.7 /100 d⁻¹ ;Gow et al., 1986). Selected White Leghorn hens had similar mean intervals between ovipositions with both continuous and Long Day lightings (24.6±0.4 and 24.8±0.2 h, respectively) whereas selected Australorps had significantly shorter interval with continuous lighting in comparison to Long Day lighting (23.0±0.2 and 23.9±0.1 h, respectively). In conclusion, the mean interval between ovipositions might be slightly altered when hens were photostimulated under Long Day or continuous lighting according to different strains and a stage of egg production.

**Hen age at photostimulation:**

Age at photostimulation is an important factor to activate the onset of reproduction in avian species (Lewis and Morris, 1998). In broiler breeders that age was advanced about 2 wk by photostimulation at either 14 or 17 wk of age compared to 20 wk of age (Yuan et al., 1994). Decreasing the age at onset of lay in broiler breeders was observed by exposing hens to photostimulation at an early age (Leeson and Summers, 1982). Early photostimulation decreased peak egg production, however, egg weight and
settable egg production were not affected (Yuan et al., 1994). Siopes (1992) reported that the number of eggs laid over 20 wk was similar when turkey hens were photostimulated at 24, 26, 28, or 30 wk of age. Poor reproductive performance has been reported in hens photostimulated at too early an age. Early lighting at 30 wk of age in Egg line turkey hens with excellent egg production (peak at 85 %) induced an arrest in laying in 50 % of hens about 3 wk after starting laying (Liu et al., 2001b). Bacon and Liu (2003) recently reported in turkey hens that a PCOF syndrome was negatively associated with hen age when hens were photostimulated with 24L:0D before 30 wk of age.

**Nutrient requirements:**

Control of body weight gain of broiler breeder females during the rearing (prelaying) and breeding periods is recently recommended and used for the production of broiler hatching eggs. Excess body weight, resulting in a failure of reproductive performance in broiler breeder hens, has been reported by McDaniel et al. (1981). Energy requirements during egg production in laying hens were reviewed by Scanes et al. (1987). Approximately 425 to 450 kcal metabolizable energy (ME) per hen per d from
2 to 3 wk before sexual maturity is needed for egg production (Waldroup and Hazen, 1976; Bornstein et al., 1979; Bornstein and Lev, 1982). During the peak egg production of broiler breeder hens, 385 kcal ME/d was adequate to maintain normal egg production (Spratt and Leeson, 1987). In broiler breeder hens the percentage of laying between 44 to 60 wk of age was substantially decreased in hens allotted 88% ME compared to 100% ME (450 kcal/d/hen; Attia et al., 1995). Leeson and Summers (2000) reported that energy intake had considerable variation in the breeder hens early in egg production (range from 220-440 kcal/hen/d). The energy requirements for a commercial strain of broiler breeder hens (Leeson and Summers, 2000) are listed below.
Comparison of calculated energy requirement (req) and feed allowance for the breeder pullets. Units are kcal ME equivalents.

<table>
<thead>
<tr>
<th>Wk of age</th>
<th>Body Weight (kg)</th>
<th>Maintenance energy req. (kcal)</th>
<th>Growth energy req.(kcal)</th>
<th>Production energy req. (kcal)</th>
<th>Total energy req.(kcal/d)</th>
<th>Highest feed energy allowance (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2.07</td>
<td>235</td>
<td>85</td>
<td>--</td>
<td>320</td>
<td>250</td>
</tr>
<tr>
<td>21</td>
<td>2.17</td>
<td>245</td>
<td>85</td>
<td>--</td>
<td>330</td>
<td>315</td>
</tr>
<tr>
<td>22</td>
<td>2.27</td>
<td>255</td>
<td>105</td>
<td>--</td>
<td>360</td>
<td>330</td>
</tr>
<tr>
<td>23</td>
<td>2.39</td>
<td>260</td>
<td>105</td>
<td>--</td>
<td>365</td>
<td>350</td>
</tr>
<tr>
<td>24</td>
<td>2.67</td>
<td>290</td>
<td>110</td>
<td>10</td>
<td>410</td>
<td>380</td>
</tr>
<tr>
<td>25</td>
<td>2.80</td>
<td>300</td>
<td>90</td>
<td>20</td>
<td>410</td>
<td>420</td>
</tr>
<tr>
<td>26</td>
<td>2.91</td>
<td>305</td>
<td>75</td>
<td>40</td>
<td>420</td>
<td>440</td>
</tr>
<tr>
<td>27</td>
<td>3.00</td>
<td>310</td>
<td>50</td>
<td>60</td>
<td>420</td>
<td>470</td>
</tr>
<tr>
<td>28</td>
<td>3.06</td>
<td>315</td>
<td>30</td>
<td>80</td>
<td>425</td>
<td>480</td>
</tr>
</tbody>
</table>

The effect of crude protein (CP) intake on reproductive performance of broiler breeder hens was evaluated by Joseph et al. (2000). Hens were fed three levels of dietary CP 14, 16, or 18 % (ME: 2.76, 2.83, 2.84 Mcal/kg, respectively) from 20 to 29 wk of age. The results showed that dietary CP did not influence body weight, body weight gain and age at sexual maturity but the duration of egg production was not sustained in the 14 % CP hens at as high a level as with the other treatments (16 and 18% CP). However, no difference in egg production over the period of 5 wk (24 to 29 wk of age)
was shown among treatments. Treatment with additional protein increased broiler breeder egg production (Brake et al., 1985). Lilburn and Myers-Miller (1990) reported that total egg production increased by feeding a high protein diet (15.5 %) during the early lay period, whereas a low protein diet (13.5 %), fed from 2 to 18 wk of age or from hatching to 21 wk of age, decreased egg production (Lilburn et al., 1987). As for ovary morphology, the number of small (between 5 to 10 mm in diameter) and larger yolky follicles (greater than 10 mm in diameter) was not different among treatments but the number of atretic large yolky follicles was greater in the treatment with 14 % CP than in the treatments with 16 or 18 % CP (0.26, 0.06, and 0.00, respectively). Some studies have showed that the requirement of protein is satisfied between 10 to 14% of feed when hens started laying (Bornstein et al., 1979; Lopez and Lesson, 1995). However, National Research Council (NRC, 1994) has reported that broiler breeder hens do not require an exact level of protein in their feed for best performance.
Management of commercial breeder hens:

The management of Arbor Acres broiler breeders is described in separate stages:

1) Brooding period (hatch to 35 d of age): The protein and energy specifications of the breeder starter should be in the range of 17.5 to 18.0% and 2800 to 2915 kcal/kg, respectively. The cumulative energy consumed is near 2500 kcal by 28 d of age. Early feed restriction is essential for good body weight control. Begin an every-other-day feeding program, or 4 & 3 program (provide feed 4 d per week and no feed on 3 d) when the feed clean up time is faster than 3 to 4 h.

2) Growing period (7 to 18 wk of age): It is recommended that the flock receive at least a small increase in weekly feed. The percentage CP and ME (kcal/kg) of feed are in a range of 15.0 to 15.5 and 2640 to 2860, respectively.

3) Pre lay period (18 to 23 wk of age): The percentage CP and ME are 15.5 to 16.5 and 2800 to 2915, respectively.

4) Laying period I (beginning at 24 wk of age): The percentage CP and ME are 15.5 to 16.5 and 2800 to 2915, respectively.

5) Laying period II (beginning at 45 to 50 wk of age): The percentage CP and ME are 14.5 to 15.4 and 2800 to 2915, respectively.
Figure 1.1  Patterns of the duration of egg production reported in various studies. The egg production rate calculated was based on the first oviposition egg of individual hens (Leghorn and Egg line and RBC3 line turkeys) and whole floor pen (Restricted-fed and full-fed broiler breeder hens).

Grossman et al., 2000  Noddegaard et al., 2000  Liu et al., 2001c
CHAPTER 2

DEVELOPMENT OF A CANNULATION PROCEDURE FOR BROILER BREEDER HENS

ABSTRACT

A cannulation and serial bleeding procedure has been developed to monitor the peripheral patterns of hormones associated with reproduction for up to 10 d in broiler breeder hens. Hens were cannulated via the jugular vein and returned to individual cages. The unrestrained cannulated hens were connected to a tether and swivel system that permitted constant infusion for maintenance of the cannula prior to serial bleeding, and unrestrained long–term serial bleeding for up to 10 d. With the procedure, a short-term experiment, with hens bled every 12 min for 36 h, and a long-term experiment, with hens bled hourly for 10 d, were conducted. In these experiments, 1.5 mL blood samples were collected at each time point using sodium citrate as the anticoagulant. To avoid hemodilution, after removal of plasma the red blood cells were reconstituted with saline to the original volume and returned to the hen of origin. Collection of serial blood samples was successful from 94% of hens in the short-term experiment, and 79% of hens in the long-term experiment. Egg production was not affected ($P>0.05$) during
the 6 wk following serial bleeding in the short-term experiment. For hens that continued laying, egg production for 10 d prior to cannulation was not different ($P > 0.05$) from egg production for the 10 d during serial bleeding in the long-term experiment. However, late in the reproductive cycle many hens stopped laying (25%) when serially bled. It was concluded that this cannulation procedure can be used to study short-term or long-term peripheral patterns of hormones associated with oviposition and ovulation in laying broiler breeder hens.

**INTRODUCTION**

In laying turkey hens, a jugular vein cannulation procedure has been developed to obtain serial blood samples for short-term or long-term monitoring of preovulatory surges of luteinizing hormone (LH) and progesterone ($P_4$) associated with spontaneous ovulations and ovipositions (Chapman et al., 1994; Yang et al., 1997, 2000; Liu et al., 2001a, Bacon et al., 2002). This cannulation and serial bleeding procedure had no adverse effects on egg production rate, and the success rate of complete blood sampling ranged between 65 and 100% for serial bleedings of 10 to 14 d in various experiments (Yang et al., 2000; Liu et al., 2001a, b, c, 2002). In turkeys, most of the problems associated with serial bleeding were due to blood clot formation at the cannula tip and associated blockage of the cannula when blood withdrawal was attempted. A secondary cause of failure was that some hens (<10%) did not adapt to cannulation, and physically damaged the cannula by pecking or scratching at the site of cannula insertion (Liu et al., 2001a, c, unpublished data).

Spontaneous ovulation and oviposition are controlled by preovulatory surges of
LH and P₄ in birds (Nakada et al., 1994; Yang et al., 1997; Bacon, et al., 2002). High resolution peripheral hormone patterns during preovulatory surges of LH and P₄ in short- and long-term studies have been reported in different lines of turkeys with different oviposition rates (Yang et al., 1997; Liu et al., 2001c, 2002). These studies show that the difference in oviposition rate is primarily associated with frequency of preovulatory surges of LH and P₄, and secondarily with the incidences of “blind” surges of LH and P₄.

In the US, about 65 million commercial broiler breeder hens are utilized annually for hatching egg production. Many studies have reported that hatching egg production is increased in broiler breeder hens by applying various nutrition restriction programs during the growing and reproduction periods (Pym and Dillion, 1974; Robbins et al., 1986, 1988; Robinson et al., 1991; Yu et al., 1992; Leeson and Summers, 2000; Nøddegaard et al., 2000). The potential differences in hormonal regulation of egg production in ad libitum-fed and restricted-fed broiler breeder hens are poorly documented to date in part due to the lack of a procedure to collect serial blood samples over several ovulation-oviposition cycles without affecting egg production. Although various procedures for serial bleeding of laying egg-type hens over a 2-d period have been reported (Joshua et al., 1977; Latimer et al., 1981; Ruschkowski et al., 1993), procedures for long-term serially sampling comparable to those developed for turkeys have not been reported.

To better document the peripheral patterns of preovulatory surges of LH and P₄ in broiler breeder hens and examine their association with differences in egg production rate due to nutritional restriction and duration of the reproductive period, a procedure to serially collect blood samples without adversely affecting ovulation and oviposition rates...
is necessary. The goal of the present study was to develop a jugular vein cannulation procedure for collection of serial blood samples from laying broiler breeder hens without adversely affecting oviposition rate. After development, the procedure was used in two studies for collection of blood samples. In the first experiment, samples were collected every 12 min for 36 h to document the peripheral patterns of preovulatory surges of LH and P₄ in laying broiler breeder hens. The results of this study are reported in Chapter 3 and in Liu et al. (2004). In the second experiment, samples were collected from broiler breeder hens given different planes of nutrition and early and late in the reproductive period every h for 10 d to determine if peripheral patterns and intervals between preovulatory LH surges are altered. The results of this experiment will be reported in Chapter 4, 5, and 6.

**MATERIALS AND METHODS**

**Cannulation and Serial Bleeding Procedure**

A training saddle (22 cm long, 22 cm wide; McMurtry and Brocht, 1984) was put on each hen 2 wk before cannulation (Fig 2.1a). The training saddle was replaced with a cannulation saddle when the hen was cannulated (Fig 2.1b). The cannulation saddle has a rubber tube (id 3mm, 15 cm length) attached to the canvas saddle for protection of the cannula (Fig 2.1b). A stainless steel spring tether (4 mm ID, 32.5 cm long¹) was fastened to the cannulation saddle with a shoe lace (Fig 2.1c). Before cannulation, feathers on a hen’s neck were clipped with scissors and the neck washed

---

¹ Instech Laboratories, Plymouth meeting, PA 19462
with 95% ethyl alcohol. At 4 sites over the right jugular vein, 1.0 mL of 2% Lidocaine\textsuperscript{2} was injected subcutaneously to induce local anesthesia. The skin over the jugular vein was opened for approximately 5 cm and the vein was visualized and dilated by digital pressure (Fig 2.1d). A portion of the vein was grasped with a hemostat to stabilize it during needle insertion. The needle used for vein puncture (16 G thin wall needle, Venocath, Venisystem\textsuperscript{3}) penetrated the skin first about 1 cm from the incision and then penetrated the vein lumen (Fig 2.1e). Successful needle insertion was indicated by bleeding through the needle. The lumen of the cannula (medical grade Silastic\textsuperscript{®} tubing, 1 m long, 0.64 mm ID X 1.19 mm OD\textsuperscript{4}) and 12 cm of the inside and outside of the cannula were treated with 1.75% TDMAC (tridodecylmethylammonium chloride heparin complex diluted with 2,2,4 tri-methyl pentane\textsuperscript{5}) for 5 min and then air dried. Treatment with TDMAC helps prevent blood clot formation at the cannula tip. The cannula was surface marked at 12, 14, and 16 cm with Sudan black dye (0.1% in toluene) and steam sterilized. The cannula was filled with sterile SCG (7.00g/L NaCl, 50g/L NaCitrate, 500 mg/L Gentamicin\textsuperscript{6}) solution, and passed through the cannulation needle and into the vein for about 12 cm. The hemostat was subsequently removed, and then the cannulation needle was removed from the vein. Bleeding was controlled by digital pressure on sterile cheesecloth, then the patency of the cannula was tested and cannula depth adjusted if necessary (Fig 2.1f). The incision was then closed with stainless steel wound staples and the cannula attached to the skin with 3 sutures. A thin wire (60 cm length) was

\textsuperscript{2} Phoenix Pharmaceutical, Inc. St Joseph MO 64503  
\textsuperscript{3} Abbott Laboratories Ltd., Dublin 24, Ireland  
\textsuperscript{4} Helix Medical, Carpinteria, CA 93013  
\textsuperscript{5} Polysciences, Inc, Warrington, PA 18976  
\textsuperscript{6} Butler, Columbus, OH 43228
connected to the end of the cannula to pull it through the rubber tube and spring tether. The end of the rubber tube was then attached to the skin with 3 loose sutures (Fig 2.1g). A patch of denim cloth (about 5 cm in length, 3 cm in width) was sutured to the skin with about 10 loose sutures to protect the exposed cannula (Fig 2.1h). A drop of super glue (Loctite, Quicktite super glue\textsuperscript{7}) was placed on all sutures. A single channel fluid swivel (18G, model 375/18\textsuperscript{8}) was attached to the end of the spring tether (Fig 2.1c) and the cannula attached.

The cannulated hen was returned to her cage, modified with a slatted rubber floor mat, and the fluid swivel was fastened to the top of the hen’s cage with a clamp attached to a wooden board (Fig 2.1i). From the fluid swivel, an extension cannula (medical grade Silastic\textsuperscript{9} tubing, 3m long, 0.76 mm ID X 1.65 mm OD\textsuperscript{9}) was attached to a 10 mL syringe and routed from the top of the cage to a syringe drive pump (Harvard model 22 multiple syringe pump\textsuperscript{10}) for infusion of SCG at a rate of 0.25 mL h\textsuperscript{-1} until serial bleeding began several days later. This system allows the cannulated broiler breeder hen freedom of movement within her individual cage (Fig 2.1j), infusion of SCG through the cannula to maintain its patency, and administration of a constant drip of the antibiotic Gentamicin\textsuperscript{\textregistered} between cannulation and initiation of serial bleeding. Several cannulated hens in their individual cages are shown in Fig 2.1k. Before starting serial blood collections, the cannula was removed from the infusion pump, and fitted with a three-way valve\textsuperscript{11}. During serial blood sample collections, the cannula was flushed into

\textsuperscript{7} Manco, Inc., Avon, OH 44011  
\textsuperscript{8} Instech labs, Plymouth Meeting, PA 18462  
\textsuperscript{9} Helix Medical, Carpinteria, CA 93013  
\textsuperscript{10} Harvard, South Natick, MA 01760  
\textsuperscript{11} Baxter, Healthcare Corporation, Deerfield, IL 60015
a 5 mL syringe containing SCG solution, the valve switched to a different syringe for collection of the 1.5 mL blood sample, and then the valve switched back to the 5 mL syringe to return flushed blood and blood remaining in the cannula to the hen with an additional 1 ml of SCG. Blood samples were transferred to 1.5 mL centrifuge tubes containing 7.5 mg sodium citrate in 15 µL saline. After centrifugation, plasma was collected and the red blood cells resuspended in saline to their original volume and put on ice. After collecting every 4 samples, the retained red blood cells were returned to the hen of origin through the cannula. An animal use protocol for these procedures was approved by the Institution Laboratory Animal Care and Use Committee (protocol #01-AG015).

**Broiler Breeder Hens and Treatments**

**Experiment 1.** This short-term serial bleeding experiment consisted of two trials. Blood samples were collected every 12 min for 36 h to test the cannulation procedure for ease of bleeding the hens and to monitor them for potential adverse effects of the procedure on egg production. Female chicks (Cobb 500\(^{12}\)) were housed in floor pens with continuous light [24 h L (light):0 h D (dark)] with *ad libitum* access to feed until 1 wk of age when they were switched to a short day photoperiod (8L:16D). This photoperiod schedule delays puberty and induces photosensitivity. At this time, a restricted feeding program, recommended by the primary breeder (Cobb 500, Management Guide), was initiated. At 10 wk of age, one-half of the hens (F) were given *ad libitum* access to feed while the other half (R) continued on the restricted

\(^{12}\) Cobb Vantress Inc., Siloam Springs, AR 72761
feeding program. At 22 wk of age, the hens were photostimulated with 16L:8D. Egg production started 2 to 3 and 3 to 4 wk after photostimulation in the F and R broiler breeder hens, respectively. The hens were placed in individual cages (32X42X46 cm) at 24 wk of age, with slatted rubber floor mats, and given continuous 24L:0D light at 28 wk of age. Continuous lighting (24L:0D) allows free running preovulatory surges of LH and P₄ throughout the 24 h solar day (Yang et al., 2000, Liu et al., 2001a, b, c, 2002).

Four F and 4 R broiler breeder hens were used in each trial. The second trial started 1 wk after the first trial. The hens were cannulated at 28 and 29 wk of age for Trials 1 and 2, respectively. The cannulas were attached to a syringe drive pump and the hens infused with SCG solution (0.25 ml h⁻¹) until serial blood sampling commenced. Serial blood samples were collected every 12 min for 36 h beginning approximately 2 wk after cannulation. After serial bleeding, 3 of 16 hens were necropsied to examine ovarian and oviductal morphology. The cannulas of the remaining 13 hens were reattached to syringe drive pumps and the hens infused at a rate of 0.25 mL h⁻¹ with SCG solution for an additional 6 wk period before necropsy. All cannulas remained patent and all hens continued to lay eggs during this 6 wk period.

**Experiment 2.** This long-term serial bleeding experiment consisted of 6 trials. At hourly intervals, 1.5 mL blood samples were collected from the hens for 10 d to test the cannulation procedure for ease of bleeding the hens and to monitor them for potential adverse effects of the procedure on egg production. Female broiler breeder chicks (Ross 508¹³) were given a 24L:0D photoperiod with *ad libitum* access to feed until 1 wk of age. Hens were then switched to a short-day photoperiod (6L:18D) until 22 wk of age when

---

¹³ Aviagen Inc., Cummings Research Park, Huntsville AL 35805
they were photostimulated with a 16L:8D photoperiod and placed in individual cages as in Experiment 1. All hens were switched to 24L:0D and infused at a rate of 0.25 mL h⁻¹ with SCG solution immediately following cannulation and 3 to 4 d before serial blood sampling began. Restricted feeding was as recommended by the primary breeder for this line of broiler breeder hens (Aviagen 2000). Hens in the 3 treatments were serially bled early (Early, peak of egg production) and late (Late, after egg production had declined by about 25%) in the reproductive period. The hens in Trials 1 (n=16) and 4 (n=16) were ad libitum–fed from hatch (FF). The hens in Trials 2 (n=17) and 5 (n=21) were given restricted access to feed until 22 wk of age and then switched to ad libitum feeding (RF). The hens in Trials 3 (n=20) and 6 (n=16) were given restricted access to feed throughout the growth and reproductive periods (RR) as recommended by the primary breeder. The hens used in each trial met the following reproduction requirement: before cannulation, all hens laid eggs for the previous 2 wk. Egg production of individual hens was recorded daily.

Statistical Analysis

Paired t-tests were used to analyze the differences (P<0.05) in body weight at cannulation and after serial bleeding, and egg production rate 10 d before cannulation and during 10 d of serial bleeding. Hens which successfully completed serial bleeding periods (no missed samples) were excluded from the study when an individual hen’s difference in egg production was more than 3 eggs between the 10 d before serial bleeding and the 10 d during serial bleeding. One way ANOVA was used to detect the difference (P<0.05) between Early and Late trials of Experiment 2 for hens successfully
serially bled, hens that kept laying during serial bleeding, and overall success rate for hens that did not stop bleeding or laying,

**RESULTS**

Body weights for the hens in each trial are given in Table 2.1. Body weight did not change in either trial in Experiment 1. In Experiment 2, hen body weight decreased during serial bleeding in Trials 1 (FF-Early), 4 (FF-Late), and 5 (RF-Late), but increased in the Trials 2 (RF-Early) and 3 (RR-Early) and did not change in Trial 6 (RR-Late).

The age of hen when cannulated and the number of hens that died during serial bleeding are given in Table 2.1. Only one hen in Trial 1 (FF-Early) of Experiment 2 died during serial bleeding. The number of hens successfully bled (no planned samples missed), the rate of successful blood sample collections, the number of hens still laying after serial bleeding, and overall success rate of serial bleeding while maintaining egg production are also shown in Table 2.1. Ovarian and oviductal morphology of the cannulated and serially bled hens in Experiment 1 were similar to non-cannulated hens (data not shown). Also, in Experiment 1 all cannulas remained patent and all hens continued to lay eggs during the 2 wk monitoring period after they were serially bled.

For Experiment 2, the trials were grouped into those Early or Late in the reproductive period. Most of the hens that stopped bleeding were in the Early trials. The rates of successful sampling for the Early and Late trials were 62±3% and 96±3% (P=0.002), respectively. Most failures in the Early trials and all failures in the Late trials were due to loss of patency of the cannula during bleeding, but some of the failures in the Early
trials were also due to physical failure of the cannula, usually due to poor protection of the cannula by the patch. The specific days during serial bleeding when a hen could no longer be bled (Table 2.2) was evenly distributed throughout the 10 d experimental period.

The hens in Experiment 2 were classified as “stopped laying” when the difference of 10 d egg production was greater than 3 eggs between the “before” and “during” bleeding periods. Four hens stopped laying before being cannulated and were removed from this study, even though complete sets of blood samples were collected from them (1 and 3 hens in the Early and Late trials, respectively). The day of last ovipositions of hens that “stopped laying” was relatively high after cannulation but before serial bleeding started in the Late trials (Table 2.3). Only one of 8 hens in Trial 5 and one of 2 hens in Trial 4 that stopped laying in the Late trials had a healthy ovary and oviduct at necropsy. The oviduct of the rest of hens that stopped laying in Trials 4 and 5 had regressed and the ovary had several atretic follicles at necropsy. All hens in Trials 3 and 6 that stopped laying had a normal ovary and oviduct with a few atretic follicles at necropsy. The rate of collecting complete sets of plasma samples from hens that kept laying was 96±6% and 75±6% (P=0.058), respectively, for the early and late groups of trials. The overall success rate (hens that kept laying and were successfully bled) was 59±4% and 71±4% (P=0.076), respectively, for the Early and Late trials.

For Experiment 1, egg production rate for the 10 d before cannulation was not different from the rate the 10 d after serial bleeding for the F (8.17 ± 1.33 and 8.17 ± 0.41 respectively, P = 1.00) and R (7.71 ± 1.11 and 7.29 ± 1.50, respectively, P = 0.48) hens. For Experiment 2, egg production rate for the 10 d before cannulation was not different
(P> 0.05) from the egg production rate during the 10 d of serial bleeding for the hens that did not stop laying in each Trial (Table 2.4). For all hens, the site of cannula insertion did not show any visual signs of infection when the patch was removed after 10 d of blood sampling (Fig 2.11) when all hens were necropsied. All hens classified as laying had a mature-size (F₁) ovarian follicle and a normal complement of hierarchical follicles when necropsied (data not shown).

DISCUSSION

Cannula insertion into the vascular system for short-term (Lestage et al., 1985; Garner et al., 1988; Tsui et al., 1991) and long-term serial blood sample collections has been widely used in physiological and pharmacologic studies in rats (Steiger et al., 1972; deJong et al., 2001) and turkeys (Chapman et al., 1994; Liu et al., 2001a, b, c, 2002). Some advantages of a cannulation system are to avoid repeated venipuncture, diminish disturbing the animal when taking serial blood samples, and less stress due to not needing to restrain animals repeatedly.

In several studies in rats, infection and lost patency of cannulas were the major factors that decreased the success rate of collection of complete sets of blood samples during long-term studies (Raad et al., 1994; Appelgren et al., 1996). Infection was the major problem in mammals during insertion of the cannula or thereafter (Goldmann and Pier, 1993). Coating the cannula with heparin has been shown to improve long term patency of cannulas and decrease lesion development in blood vessels in rats because it has anti-thrombotic and anti-microbial functions (Arnander et al., 1987; Appelgren et al., 1996, Foley et al., 2002). A self-powered constant infusion device, used to deliver
drugs, maintains intravascular cannula patency, prevents and delays thrombus formation, and prolongs a period of bleeding in unrestrained rats (Brand et al., 2000).

In the current studies with broiler breeder hens, infection was of minor importance, since visual signs of infection and inflammation were not found at the site of cannula insertion after 10 d of serial bleeding (Experiment 2) or at 6 wk following serial bleeding (Experiment 1). The data showed that most laying hens (38%) that lost cannula patency were in the Early rather than the Late trials. Most cases of lost cannula patency were probably due to blood clot formation in the jugular vein independent of infection. In these hens, SCG could be infused into the vein via the cannula, but blood could not be withdrawn. In a few cases, stretching the hen’s neck allowed continued blood sample collection, but these hens would often quit bleeding 1 or 2 days later. For unknown reasons, the Early hens had a higher incidence of blood clot formation in the vein during long-term bleeding than the Late hens.

After 10 d of serial bleeding (Experiment 2), body weight decreased in FF-Early (-6%) and Late (-3%) hens (Trials 1 and 4), suggesting the appetite of the FF hens was lower during serial bleeding, resulting in a decrease in body weight. In comparison, body weight of RF-Early (Trial 2) and RR-Early (Trial 3) hens increased (+5% and +6%, respectively) early in the reproductive period when the hens in these groups were still gaining weight due to increased feed allocation at 22 wk of age. Increasing body weight in the RF-Early hens (Trial 2) might be due to compensatory growth as this was only 7 to 8 wk after switching to *ad libitum* from restricted-fed feeding. Body weight of the RF-Late hens decreased (-4%) late in the reproductive period (Trial 5) after compensatory growth had occurred, and the decrease was of similar
magnitude as for the FF-Late hens.

Only one of the 106 hens cannulated and serially bled (Trial 1) died during long-term serial bleeding. This suggests that short- and long-term serial bleeding has little effect on survival rate of the hens during serial bleeding.

The success rate of complete serial bleeding increased late in comparison to early in the reproductive period but more hens stopped laying during sampling late in the reproductive period. Previous experience with turkey hens and current experience with broiler breeder hens suggests that hens late in the reproductive period are more sensitive to the adverse effects of cannulation and 10 d of serial bleeding because a higher percentage of hens stopped laying (Liu et al., 2001c, unpublished and current data). It is unclear whether the increase in the rate of hens that stopped laying in Experiment 2 late trials was because of possible stress associated with cannulation and serial bleeding or due to natural ageing, resulting in both a decreased rate of egg production and an increased rate of cessation of egg production. In turkey studies, fewer small turkeys with a good rate of egg production stopped laying during long-term serial bleeding in comparison to large turkeys with a poor rate of egg production both early and late in the reproductive period (Liu et al., 2001c, 2002, unpublished data). In Trial 5 of Experiment 2, the RF-Late hens had the highest percentage (8/21) of hens that stopped laying late in the reproductive period for unknown reasons. The current studies with broiler breeder and earlier turkey studies (Liu et al, 2001a, b, c, 2002) suggest that duration of the reproductive period is positively associated with the percentage of hens that stopped laying during long-term serial bleeding. The effect of body weight on the percentage of hens that kept laying during cannulation and serial bleeding appears to be
less important than duration of egg production.

In conclusion, the cannulation procedure for short-term serial bleeding (samples collected every 12 min for 36 h) of broiler breeder hens had a high success rate for collection of complete sets of blood samples, and did not affect egg production during the 10 d before cannulation in comparison to the 10 d after cannulation. During long-term serial bleeding (samples collected hourly for 10 d), less than 10% of broiler breeder hens ceased laying early in comparison to 25% of hens late in the reproductive period, but cannula patency was lost in more hens early (37%) than late (4%) in the reproduction period during long term serial bleeding. Thus, this cannulation procedure can be used to study short-term or long-term changes in reproductive hormone concentration associated with oviposition and ovulation in laying broiler breeder hens with an overall success rate of about 94% and 65%, respectively.

REFERENCES


<table>
<thead>
<tr>
<th>Variable</th>
<th>Experiment 1&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Experiment 2&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1 (FF-Early)</td>
<td>Trial 2 (RF-Early)</td>
</tr>
<tr>
<td>Age when cannulated (wk)</td>
<td>28-29</td>
<td>28-29</td>
</tr>
<tr>
<td>Hens cannulated (n)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Body weight before cannulation (kg)</td>
<td>5.45±0.36</td>
<td>3.46±0.27</td>
</tr>
<tr>
<td>Body weight after bleeding (kg)</td>
<td>5.34±0.40</td>
<td>3.47±0.34</td>
</tr>
<tr>
<td>Difference in body weight (&lt;P ≤)</td>
<td>0.30</td>
<td>0.84</td>
</tr>
<tr>
<td>Hens died during bleeding (n)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hens successfully bled (n)</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Hens successfully bled (%)</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Hens laying after serial bleeding (n)</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Hens laying after serial bleeding (%)</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Overall success (n)</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Overall success (%)</td>
<td>100</td>
<td>88</td>
</tr>
</tbody>
</table>

<sup>1</sup> Hens serially bled every 12 min for 36 h.

<sup>2</sup> Hens serially bled every h for 240 h.

<sup>3</sup> Hens successfully serial bled that continued laying after (Exp 1) or during (Exp 2) serial bleeding.

Table 2.1. Summary of cannulation system results.
Table 2.2. Distribution of when cannula patency was lost during 10 d of serial bleeding (hourly samples for 10 d) in Experiment 2.

<table>
<thead>
<tr>
<th>Trials</th>
<th>Hens (n)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹Early (1, 2, and 3)</td>
<td>53</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>²Late (4, 5, and 6)</td>
<td>53</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

¹ Hens were between 25 and 33 wk of age.
² Hens were between 43 and 59 wk of age.
Table 2.3. Distribution of when hens stopped laying (d of last oviposition) after cannulation or during serial bleeding in Experiment 2.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Hens (n)</th>
<th>Serial bleeding day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-2</td>
</tr>
<tr>
<td>Early (1, 2, and 3)</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>Late (4, 5, and 6)</td>
<td>53</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Hens were between 25 and 33 wk of age.
2 Hens were between 43 and 59 wk of age.
Table 2.4. Egg production (n) of the hens that did not stop laying for the 10 d before cannulation and the 10 d during serial bleeding of broiler breeder hens in Experiment 2.

<table>
<thead>
<tr>
<th>Egg production¹ (%)</th>
<th>Trial 1 (FF-Early)</th>
<th>Trial 2 (RF-Early)</th>
<th>Trial 3 (RR-Early)</th>
<th>Trial 4 (FF-Late)</th>
<th>Trial 5 (RF-Late)</th>
<th>Trial 6 (RR-Late)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>7.11±0.93</td>
<td>8.70±1.16</td>
<td>8.64±1.12</td>
<td>5.50±1.83</td>
<td>5.54±1.71</td>
<td>6.08±0.90</td>
</tr>
<tr>
<td>During</td>
<td>7.33±1.50</td>
<td>8.40±1.71</td>
<td>8.00±0.62</td>
<td>5.33±1.83</td>
<td>5.15±1.77</td>
<td>5.42±1.73</td>
</tr>
<tr>
<td>P</td>
<td>0.65</td>
<td>0.56</td>
<td>0.09</td>
<td>0.75</td>
<td>0.56</td>
<td>0.12</td>
</tr>
</tbody>
</table>

¹ Data are for hens that did not stop egg production and were successfully serial-bled.
Figure 2.1: Cannulation in laying broiler breeder hens.  a. Training saddle.  b. Cannulation saddle.  c. Cannulation saddle connected to a spring and tether.  d. A visible right jugular vein.  e. A jugular vein successfully penetrated by a thin wall cannulation needle.  f. Cannula inserted into the vein and tested for bleeding.  g. Incision closed with stainless steel wound staples and the cannula and rubber tube attached to the skin with loose sutures and super glue.  h. Denim cloth patch attached to the skin with loose sutures to protect cannula.  i. Swivel attached at top of the cage.  j. Spring and tether attached on swivel to allow the hen free movement in the cage.  k. Several canulated hens in individual cages ready for serial blood sampling.  l. Site of cannulation after serial bleeding for 10 d.
CHAPTER 3

PREOVULATORY SURGE PATTERNS OF LUTEINIZING HORMONE, PROGESTERONE, AND ESTRADIOL-17\(\beta\) IN AD LIBITUM-FED AND RESTRICTED-FED BROILER BREEDER HENS

ABSTRACT

Spontaneous ovulations are induced by preovulatory surges of luteinizing hormone (LH) and progesterone (P₄) during ovulatory cycles in birds, while estradiol-17\(\beta\) (E₂) levels are relatively constant. Egg production is enhanced in restricted-fed (R) in comparison to ad libitum-fed (F) broiler breeder hens, but changes in concentrations and peripheral patterns of LH, P₄, and E₂ during ovulatory cycles in broiler breeder hens are poorly documented. The hypothesis of this study was that high resolution patterns of peripheral LH, P₄, and E₂ during preovulatory surges would not be different between F and R broiler breeder hens. Seven F and 6 R broiler breeder hens were photostimulated with 16 h L (light):8 h D (dark) at 22 wk of age. At 28 wk of age, the hens were cannulated for serial blood sampling and switched to a 24L:0D photoperiod to allow preovulatory surges of LH and P₄ to free run. Three days after cannulation, hens were serially bled every 12 min for 36 h. The F hens were heavier than the R hens (5.60±0.35 vs
3.60±0.28 kg, respectively; $P<0.01$). During the 10 d before cannulation, total egg production of the F and R hens (8.3±1.4 and 6.8±1.3 eggs, respectively; $P=0.08$) and normal egg production (5.6±1.8 and 6.5±1.8 eggs, respectively; $P=0.37$) were not different. The F hens however, had a higher number of abnormal eggs than the R hens (2.7±1.7 and 0.3±0.8 eggs, respectively; $P<0.01$). None of the hormonal measurements were different between the F and R hens ($P>0.05$). The concentrations of hormones, respectively, for the F and R hens were: baseline LH (2.79±0.45 vs 2.94±0.60 ng mL$^{-1}$) and $P_4$ (1.68±0.56 vs 1.41±0.43 ng mL$^{-1}$), overall mean LH (3.18±0.45 vs 3.10±0.46 ng mL$^{-1}$) and $P_4$ (2.32±0.55 vs 2.09±0.91 ng mL$^{-1}$), preovulatory surge amplitude of LH (5.43±1.27 vs 3.88±1.24 ng mL$^{-1}$) and $P_4$ (6.08±2.09 vs 6.71±3.91 ng mL$^{-1}$), preovulatory surge duration of LH (7.52±1.80 vs 5.74±3.18 h) and $P_4$ (7.52±1.42 vs 8.20±1.24 h), and overall mean $E_2$ (0.25±0.05 vs 0.23±0.05 ng mL$^{-1}$). In conclusion, there were no differences in total egg production or normal egg production between F and R broiler breeder hens, but the F hens laid more abnormal eggs. Also, there were no differences in the concentrations or peripheral patterns of LH, $P_4$, and $E_2$ during preovulatory surges between the F and R broiler breeder hens.

**INTRODUCTION**

It is well accepted that spontaneous ovulation is associated with preovulatory surges of luteinizing hormone (LH) and progesterone ($P_4$) during ovulatory cycles in Japanese quail (Doi et al., 1980), ducks (Tanabe et al., 1980; Wilson et al., 1982), egg-type chickens (Furr et al., 1973; Johnson and van Tienhoven, 1980; Etches and Cheng, 1981), and turkeys (Chapman et al., 1994; Liu et al., 2001a, b, c). Short- and
long-term peripheral patterns of LH and P₄ during preovulatory surges have been described in detail in laying turkey hens (Yang et al., 1997, 2000; Liu et al., 2001a, b, c, 2002). High resolution peripheral patterns of LH and P₄ during preovulatory surge, and of estradiol-17β (E₂) have not been as well documented in laying chicken hens, however, due to the lack of a robust procedure for serial blood sampling. The data published for laying chicken hens are derived from blood samples collected by venipuncture from hens sampled every 2 to 6 h for up to 30 h during ovulatory cycles. Recently, a cannulation and serial bleeding procedure for broiler breeder hens has been developed (Liu et al., 2004).

Spontaneous ovulations and ovipositions are hormonally controlled in birds. Ovulation of a single follicle in laying chicken hens, or ovulation of multiple follicles after hypophysectomy of laying chicken hens, can be induced by exogenous injection of LH (Opel and Nalbandov, 1961a, b). The injection of P₄ has both acute and chronic effects. Premature ovulation can be induced in laying chicken hens by an acute subcutaneous injection of P₄ (Fraps and Dury, 1943). Laying turkey hens, however, given daily subcutaneous injections of P₄ may develop a polycystic ovarian follicle syndrome associated with blocked spontaneous ovulations and ovipositions (Bacon and Liu, 2004), while laying chicken and quail hens stop laying within a few days but do not develop the polycystic ovarian follicle syndrome (Liu and Bacon, submitted).

The injection of E₂ induces the production of yolk precursor lipoproteins by the liver, stimulates oviductal development, and modifies calcium metabolism in laying hens (Walzem, 1996; Chen et al., 1999; Walzem et al., 1999; Johnson, 2000). Studies in laying turkey hens have shown that peripheral patterns of E₂ do not follow a peripheral
pattern similar to the preovulatory surges of LH and P₄ (Liu et al., 2001c, 2002, Bacon et al., 2002), but are relatively constant. Several studies in chickens (Lague et al., 1975) and Japanese quail (Doi et al., 1980) hens have shown that E₂ is not effective in induction of ovulations.

In turkey hens, baseline LH increases 1 to 2 d after photostimulation and is maintained at a high level until egg production starts to decline (Bacon and Long, 1995, 1996; Yang et al., 1999). Baseline P₄ increases steadily from undetectable concentrations during the 4 d before first LH preovulatory surges to a level between 1.0 and 2.0 ng/ml during the egg production period (Liu et al., 2002; Bacon et al., 2002). E₂ increases 4 to 6 d after photostimulation, and then does not change much during the egg production period in turkey hens (Liu et al., 2002; Bacon et al., 2002). Several hundred preovulatory surges of LH and P₄ were examined in laying turkey (Liu et al., 2001a, b, c, 2002; Yang et al., 2000; Bacon et al., 2002) and chicken hens (Furr et al., 1973; Etches and Cunningham, 1976; Proudman et al., 1984) and in all cases, LH preovulatory surges were coupled with P₄ preovulatory surges. In different studies where blood samples were collected at various intervals, single preovulatory surges of LH and P₄ were observed 4 to 8 h before oviposition in laying chicken hens (Furr et al., 1973; Shodono et al., 1975; Mashaly et al., 1976; Williams and Sharp, 1978). In cannulated laying turkey hens serially sampled every 10 min for 26 h, the concentrations of LH increased to a peak over a 2 to 3 h period during preovulatory surges and then declined to baseline concentrations over the ensuing 4 to 6 h (Yang et al., 1997), while preovulatory surges of P₄ increased over 1 to 2 h to a plateau peak lasting about 6 h before declining to baseline over 1 to 2 h (Yang et al., 1997). Thus, the duration of P₄ surges was 2 to 3 h longer.
than LH preovulatory surges. Also, the initiation of P4 preovulatory surges was coincident with the initiation of the LH preovulatory surges (Yang et al., 1997).

It has been known for many years, that broiler breeder females are at a reproductive disadvantage when they are allowed ad libitum-feed (Jaap and Muir, 1968; Hocking et al., 1987; Robinson et al., 1993). Excess body weight, especially body fat, is associated with reduced reproductive efficiency of ad libitum-fed hens (McDaniel et al., 1981; Pearson and Herron, 1981). Restricting feed intake during growth and reproduction therefore, is practiced for broiler breeder hens to achieve better reproductive efficiency (Yu et al., 1992; Bruggeman et al., 1999; Leeson and Summers, 2000; Nøddegaard et al., 2000). Although restricted feeding delays the onset of sexual maturity, it can result in increased reproductive efficiency by increasing total egg production, improving fertility and hatchability, decreasing the incidence of double-yolked and soft-shell eggs, and lowering the rate of mortality (Hocking et al., 1987, 1989; Yu et al., 1992; Hocking 1993).

There have been no detailed, comparative endocrine relationships established for ad libitum-fed versus restricted-fed broiler breeder hens. The objective of the current study therefore, was to determine the detailed peripheral patterns of LH, P4, and E2 during the preovulatory surge in ad libitum-fed and restricted-fed laying broiler breeder hens early in the reproductive period production of eggs suitable for incubation was expected to be lower in the ad libitum-fed hens. The hypothesis of this study was that high resolution patterns of peripheral LH, P4, and E2 during preovulatory surges would not be different between ad libitum-fed versus restricted-fed broiler breeder hens.
MATERIALS AND METHODS

Animals and Blood Sample Collection

One-day-old broiler breeder hens (Cobb 500\textsuperscript{1}) were raised in floor pens with continuous light [24 h light (L):0 h dark (D)] and with \textit{ad libitum} access to feed until 1 wk of age. All of the hens were given restricted access to feed from 1 to 10 wk of age as recommended by the supplier (Cobb 500, Management Guide). At 10 wk of age, one-half of the hens (n=7) were given \textit{ad libitum} access to feed (F) and the other one-half (n=7) continued to be given restricted access to feed (R) as recommended by the supplier. As recommended by the supplier the hens were provided with a 6L:18D photoperiod from 1 to 22 wk of age, when they were photostimulated with a 16L:8D photoperiod to induce egg laying. The hens were moved into individual cages (32X42X46 cm) with slatted rubber floor mats at 24 wk of age. Egg production started 2 to 3 wk and 3 to 4 wk after photostimulation in F and R hens, respectively. At 28 wk of age and prior to cannulation, they were switched to 24L:0D to allow preovulatory surges of LH and P\textsubscript{4} to free run without any masking effects of diurnal lighting. Egg production was recorded daily before cannulation and during serial blood sampling, and the hens were digitally palpated 3 times a day to identify the presence of a soft or hard shell egg in the uterus of the oviduct. The hens were cannulated and serially bled between 28 and 29 wk of age. The procedure for serially bleeding of broiler breeder hens is described in detail in a separate communication (Liu et al., 2004). Briefly, after jugular vein cannulation, the hens were returned to their individual cages and connected to a swivel and tether system with an extension cannula and placed on a continuous infusion of 0.7% NaCl, 0.5% Na

\textsuperscript{1}Cobb Ventress Inc, Siloam Springs, AR. 72761
citrate, 0.5 mg/mL Gentamicin at a rate of 8 mL/d. This allowed cannulated hens free movement within their cage during serial blood sample collection. Serial blood sample collection consisted of collecting 1.5 mL blood samples every 12 min for 36 h, using Na citrate (5 mg/mL blood) as anticoagulant. After centrifuging the blood samples, the plasma was removed and stored at -20° for hormone assays. The red blood cells were suspended to original blood volume with sterile saline and returned to the individual hen of origin after every 3 to 4 samples to avoid hemodilution (Chapman et al., 1994). The body weight of F and R hens was recorded before cannulation and after serial blood sampling. An animal use protocol for these procedures was approved by the Institutional Laboratory Animal Care and Use Committee (Protocol #01-AG015).

LH, P₄, and E₂ Assays

Concentrations of LH were measured by radioimmunoassay (RIA) as previously described using 100 µL plasma for each duplicate (Bacon and Long, 1996). Blood samples collected every 12 min for 36 h were assayed. The intrassay coefficients of variation (CV) of plasma pools from laying broiler breeder hens with high (4.59 ng/mL) or low (3.55 ng/mL) concentrations of LH were 6.3% and 5.1%, respectively. The interassay CV for these pools were 10.0% and 11.5%, respectively. The concentration of P₄ was measured by RIA using 12 µL of plasma for each duplicate (Yang et al., 1997). Blood samples collected every 24 min for 36 h were assayed. The intraassay and interassay CV of a plasma pool (5.02 ng/mL) from laying broiler breeder hens were 11.2% and 10.8%, respectively. The concentration of E₂ was determined in a single RIA using 100 µL plasma for each duplicate on samples collected every 3 h by the
method of Chen et al. (1999). The intraassay CV of a pool of plasma with high (mean=0.43±0.05 ng/ml) or a low (mean=0.35±0.01 ng/mL) E2 concentrations were 11.0% and 4.0%, respectively.

**Statistical Analyses**

The LH and P4 raw data were evaluated by the Pulsar algorithm to identify LH and P4 preovulatory surges, and to calculate baseline, overall, and peak amplitude concentrations, and preovulatory surge durations of LH and P4 (Merriam and Wachter, 1982). For both LH and P4, the G-values used for Pulsar analysis were G(1)=50, G(2)=2.6, G(3)=1.9, G(4)=1.5, G(5)=1.2. The standard deviations for LH and P4 used for PULSAR analyses were (11.5X)/100 and (11.2X)/100, respectively, where X is the sample concentration measured in an individual sample. The following measurements were analyzed by one way ANOVA between the R and F broiler breeder hens as main effect and hens as error term: 1) body weight before cannulation and after serial bleeding; 2) number of eggs laid during the 10 d before serial bleeding; 3) number of normal eggs laid during the 10 d before serial bleeding; 4) number of abnormal eggs laid during the 10 d before serial bleeding; 5) overall mean concentration of LH, P4, and E2; 6) baseline concentrations of LH and P4; and 7) peak amplitude concentrations of LH and P4; and 8) duration of preovulatory surges of LH and P4.

**RESULTS**

A complete set of serial blood samples (1.5 ml every 12 min for 36 h) was obtained from 7 of 7 F hens and from 6 of 7 R broiler breeder hens. During the serial
bleeding period, one R hen stopped bleeding and was removed from the study. Complete preovulatory surges of LH and P₄ were retrospectively observed in 5 of 7 F and 3 of 6 R hens.

The F hens were heavier than the R hens (Table 3.1). During the 10 d period before cannulation, total and normal egg production of the F and R hens were not different (Table 3.1). The F hens however, had a higher number of abnormal eggs than the R hens. Representative peripheral patterns of LH (every 12 min), P₄ (every 24 min), and E₂ (every 3 h) over 36 h for F and R broiler breeder hens during the occurrence or absence of LH and P₄ preovulatory surges are shown in Figure 3.1. Similar patterns of preovulatory LH and P₄ surges, and E₂ were observed for the F and R hens. Oviposition time was not strongly coincident with the declining concentration of P₄ during the ovulatory cycles (Fig 3.1). The overall mean, baseline, and preovulatory surge amplitude concentrations of LH and P₄ were not different between F and R hens (Table 3.2) nor were the overall mean concentrations of E₂.

Each P₄ preovulatory surge was coincident with a preovulatory surge of LH, but not E₂ (Figure 3.1a, c) and the duration of preovulatory LH and P₄ surges were not different between F and R hens (Table 3.3). The concentrations of LH during a preovulatory surge increased over 2.5 h and 1.5 h from the baseline levels to the peak levels and then declined over 4.9 h and 4.0 h to baseline levels for the F and R hens, respectively (Table 3.3). Thus, the duration of the ascending limb of the preovulatory surge accounts for about one-third, and the descending limb about two-thirds of the duration of the LH preovulatory surge. Preovulatory surges of P₄ increased slightly before or at the same time as LH preovulatory LH surges, but were maintained longer and
with a broader plateau than the LH preovulatory surges (Figure 3.1a, c and Table 3.3). The duration of the ascending limb of P4 preovulatory surges is similar to that of the descending limb in both F and R hens (Table 3.3). The concentrations of LH and P4 were maintained at relatively stable baseline levels between preovulatory surges (Figure 3.1b and d). Oviposition occurred when P4 concentrations had declined following the preovulatory surge (Fig 3.1a, c) or had returned to a baseline level between preovulatory surges (Fig 3.1b, d). For the 36 h period observed, additional small peaks of LH were not observed prior to or after the preovulatory surges (Fig 3.1b, d). Increased E2 concentrations were not always coincident with LH and P4 preovulatory surges during ovulatory cycles.

**DISCUSSION**

Restricted feeding of broiler breeder hens is known to delay sexual maturity but to increase total egg production and settable egg production over the entire reproductive cycle. In the current study, egg production started 3 to 4 wk after photostimulation in R hens and increased to peak production in about 3 wk whereas F hens reached an earlier peak in egg production, in agreement with Nøddegaard et al. (2000). Egg production for 10 d before serial blood collection was not different between the R and F hens, but abnormal egg production was higher in the F hens, suggesting that lower egg production usually measured in *ad libitum*-fed broiler breeder hens under floor housing conditions (Yu et al., 1992) may be due to the production of abnormal eggs which may be lost and not recorded for floor housed hens.

It has been reported that occurrence of preovulatory surges of LH in laying
hens was restricted to late in scotophase and early in photophase under diurnal photostimulation (Yang et al., 2000). In the current study, continuous lighting was used to facilitate blood sample collection, but this allows preovulatory surges of LH and P4 to free run. Thus, surges of these hormones were expected to occur anytime during serial sampling. Collection of blood samples during entire preovulatory surges of LH and P4 were obtained from a lower number of hens than projected because partial preovulatory surges of LH and P4 were observed in more hens than expected, and no surges were observed in other hens during the 36 h of blood sampling. The lack of LH surges did not affect the estimation of baseline LH or P4 concentrations, but probably led to an underestimation of the overall LH concentration. The lack of surges also led to the low number of observations for both LH and P4 surge amplitude concentrations and duration of the surges. No additional hens were available for sampling, so a more accurate examination of potential effects of restricted feeding on LH and P4 surge amplitude concentrations and duration of the surges must await future studies, which are currently underway (Liu and Bacon, unpublished)

Two ovipositions, one of a normal hard shell egg and one of a soft shell egg, were found in a 6 h period during serial bleeding in one of the F hens. The preovulatory surges of LH and P4 related to these two eggs were not observed, however, because they occurred before initiation of serial bleeding of the hen. It was observed, however, that the first egg was laid before the observed preovulatory surges of LH and P4 and the second, a soft shelled egg, was laid just after the LH and P4 concentrations had returned to baseline levels. Ovipositions may also occur when P4 is at the baseline level between ovulatory surges, or when P4 is still at it’s surge concentration. These observations
suggest that ovipositions might not be tightly associated with the P₄ concentration during ovulatory cycles.

The characteristics of preovulatory surges of LH in F and R broiler breeder hens were similar to those of turkey hens in that LH concentrations were maintained at relatively stable baseline levels outside of peak surges (Yang et al., 1997; Liu et al., 2001a, b, c, 2002). Etches and Cheng (1981) reported that an additional small peak of LH occurred at 14 to 16 h before spontaneous ovulations in egg-type chicken hens. In the current study with broiler breeder hens under constant lighting, or in turkey hens with both diurnal and constant lighting (Yang et al., 1997; Liu et al., 2001a, b, c, 2002), this additional small peak was not observed. This small peak reported by Etches and Cheng (1981) coincided with the start of the photophase, which was absent in the current study. Also, the hens were serially bled in the current study rather than bled by veinipuncture. These differences in experimental protocol may have contributed to the absence of this small LH peak in the current study. Also, the current data suggest that this small peak is not necessary for spontaneous ovulation in laying chicken hens as well as in laying turkey hens.

The duration of LH and P₄ preovulatory surges was not different between the F and R broiler breeder hens, suggesting the preovulatory surge duration is not an important factor contributing to the differences in egg production, including laying of abnormal eggs. The current study also agrees with turkey studies in that preovulatory surge duration of LH was not different when body weight and egg production rates were different early or late in the reproductive period (Liu et al., 2001c, 2002, unpublished data). In turkey hens however, poor egg production rates were largely associated with
longer intervals between preovulatory surges intervals of LH (Liu et al., 2001c, 2002). The intervals between preovulatory surges of LH in relation to total egg and abnormal egg production in broiler breeder hens have not yet been studied.

The data suggest that P4 preovulatory surges begin slightly earlier or at the same time as LH preovulatory surges in broiler breeder hens. This is in agreement with studies with blood sampling over hourly intervals in turkeys (Mashaly et al., 1976; Yang et al., 1997), chickens (Kappauf and van Tienhoven, 1972; Duplaix et al., 1981; Proudman et al., 1984), ducks (Tanabe et al., 1980; Wilson et al., 1982), and the Japanese quail (Doi et al., 1980) which show that the preovulatory surges of LH increase at the same time or slightly earlier than the P4 surges.

Not like some mammalian species (i.e., pig: Stickan et al., 1999), where E2 surges occur before preovulatory LH surges, no change in E2 level was detected in laying broiler breeder hens in association with LH and P4 surges. The concentration of E2 was relatively constant during ovulatory cycles in all broiler breeder hens during serial blood sampling for 36 h whether or not surges of LH occurred. This is in agreement with the data from laying turkey hens (Bacon et al., 2002) and suggests that peripheral changes in E2 level are not associated with preovulatory LH and P4 surges.

In conclusion, in both R and F broiler breeder hens, preovulatory surges of LH and P4 were associated with spontaneous ovulations, but surges of E2 were not detected. In addition, the difference in abnormal egg production induced by different feeding programs for broiler breeder hens was not associated with differences in baseline and preovulatory surge amplitude concentrations of LH and P4, duration of preovulatory surges of LH and P4, or overall concentration of E2. The characteristics of preovulatory
surges of LH and P₄ in R hens were similar to those in F hens. Interval between preovulatory surges of LH and P₄ is one candidate factor that may contribute to a decrease in egg production rate in *ad libitum*-fed hens, and is described in Chapter 4.

REFERENCES


Duplaix, M., J. Williams, and P. Mongin, 1981. Effects of an intermittent lighting schedule on the time of egg-laying and the levels of luteinizing hormone, progesterone and corticosterone in the plasma of the domestic hen. J. Endocrinol. 91:375–383.


Kappau, B., and A. van Tienhoven, 1972. Progesterone concentrations in peripheral plasma of laying hens in relation to the time of ovulation. Endocrinology


<table>
<thead>
<tr>
<th>Treatment</th>
<th>BW (kg)</th>
<th>Egg production&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before&lt;sup&gt;1&lt;/sup&gt;</td>
<td>After&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>F (n=7)</td>
<td>5.60±0.35</td>
<td>5.50±0.38</td>
</tr>
<tr>
<td>R (n=6)</td>
<td>3.60±0.28</td>
<td>3.56±0.38</td>
</tr>
<tr>
<td><em>P</em> ≤</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1. Body weight before cannulation.
2. Body weight after blood sample collection.
3. 10 d before cannulation.
4. Abnormal eggs = soft-shelled, extra calcified, double yolked, and broken eggs.

Table 3.1. Body weight and egg production of *ad libitum* -fed (F) and restricted-fed (R) broiler breeder hens.
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Treatment</th>
<th>Overall mean (ng/mL)</th>
<th>Baseline (ng/mL)</th>
<th>Peak amplitude (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH(^1)</td>
<td>F</td>
<td>3.18±0.45 (7)(^4)</td>
<td>2.79±0.45 (7)</td>
<td>5.43±1.27 (5)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>3.10±0.46 (6)</td>
<td>2.94±0.60 (6)</td>
<td>3.88±1.24 (3)</td>
</tr>
<tr>
<td></td>
<td>(P \leq)</td>
<td>0.81</td>
<td>0.64</td>
<td>0.14</td>
</tr>
<tr>
<td>P(_4)^2</td>
<td>F</td>
<td>2.32±0.55 (7)</td>
<td>1.68±0.56 (7)</td>
<td>6.08±2.09 (5)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.09±0.91 (6)</td>
<td>1.41±0.43 (6)</td>
<td>6.71±3.91 (3)</td>
</tr>
<tr>
<td></td>
<td>(P \leq)</td>
<td>0.58</td>
<td>0.36</td>
<td>0.76</td>
</tr>
<tr>
<td>E(_2)^3</td>
<td>F</td>
<td>0.25±0.05 (7)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>0.23±0.05 (6)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>(P \leq)</td>
<td>0.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) LH concentrations were measured every 12 min for 36 h.
\(^2\) P\(_4\) concentrations were measured every 24 min for 36 h.
\(^3\) E\(_2\) concentrations were measured every 3 h for 36 h.
\(^4\) Number of observations.

Table 3.2. Concentrations of luteinizing hormone (LH), progesterone (P\(_4\)), and estradiol-17\(\beta\) (E\(_2\)) early in the reproductive period of ad libitum-fed (F) and restricted-fed (R) broiler breeder hens.
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Treatment</th>
<th>n</th>
<th>Total duration (h)</th>
<th>Baseline to peak(^1) (h)</th>
<th>Peak to baseline(^2) (h)</th>
<th>(P \leq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>F</td>
<td>5</td>
<td>7.52±1.80</td>
<td>2.52±1.47 (33.9)(^3)</td>
<td>4.92±1.78 (66.0)(^3)</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>3</td>
<td>5.74±3.18</td>
<td>1.53±0.46 (27.7)</td>
<td>4.00±2.96 (72.3)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>(P \leq)</td>
<td></td>
<td>0.34</td>
<td>0.32</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>P(_4)</td>
<td>F</td>
<td>5</td>
<td>7.52±1.42</td>
<td>3.52±0.95 (46.8)</td>
<td>4.00±0.57 (53.2)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>3</td>
<td>8.20±1.24</td>
<td>4.27±1.29 (48.5)</td>
<td>4.53±1.67 (51.5)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>(P \leq)</td>
<td></td>
<td>0.48</td>
<td>0.40</td>
<td>0.52</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Duration of increase from baseline to peak during a preovulatory surge.

\(^2\) Duration of decline from peak to baseline during a preovulatory surge.

\(^3\) Percentage of duration associated with increase or decline during surges.

Table 3.3. Duration of preovulatory surges of luteinizing hormone (LH) and progesterone (P\(_4\)), early in the reproductive period of *ad libitum*-fed (F) and restricted-fed (R) broiler breeder hens.
Figure 3.1. Peripheral patterns of luteinizing hormone (LH), progesterone (P₄), and estradiol-17β (E₂) in plasma of ad libitum-fed (F; a, b) or restricted-fed (R; c, d) broiler breeder hens at 12 min (LH), 24 min (P₄), or 3 h (E₂) intervals for 36 h. Four representative hormone profiles from individual hens (# = hen badge) with and without a preovulatory surges of LH and P₄ in F (a, b) and R (c, d) hens are presented. Closed circles at top of each panel represent time of oviposition (ov) of eggs based on palpation and egg collection records. The open circle at top-middle of panel a represents an oviposition of a soft-shelled (SS) egg (a). Open circles at the top-right corner of panels a and c represent a soft-shelled egg in the uterus at the end of serial bleeding. Arrows give assigned association of the preovulatory surges of LH and P₄ to an oviposition based on palpation and oviposition records. Each P₄ preovulatory surge was coincident with an LH preovulatory surge (a and c).
CHAPTER 4

PREOVULATORY LUTEINIZING HORMONE SURGE INTERVAL OF BROILER BREEDER HENS INCREASES WITH DURATION OF THE REPRODUCTIVE PERIOD BUT IS NOT CHANGED BY FEED RESTRICTION

ABSTRACT

Feed restriction of broiler breeder hens during growth and reproduction periods is associated with an increase in settable egg production. Egg production peaks 3 to 6 wk after initiation, then declines 1.0 to 1.5 % wk⁻¹. It was hypothesized that feed restriction does not alter the frequency of LH surges, but that the frequency of LH surges declines with progression of the reproductive period. Hens were reared with ad-libitum (F) or restricted (R) feeding. When photostimulated at 22 wk of age, hens continued to be ad-libitum (FF), or restricted fed (RR), or were switched from restricted to ad libitum feeding (RF). Blood samples were collected hourly at peak of production (Early, 3 to 6 wk) and after production had declined by about 25% (Late, 20 to 30 wk). Total egg
production for the FF, RF, and RR groups was 74%, 84%, and 76% (P=0.397), for Early hens and 53%, 52%, and 56% (P=0.931) for Late hens, respectively during serial bleeding. Total egg production was higher for Early than Late hens (P<0.001). Production of abnormal eggs was not different between the Early-FF (16.3%) and Early-RF (9.4%) hens, but was lower (P<0.05) in the Early-RR (0.0%) in comparison to Early-FF hens. Production of abnormal eggs was higher in Late-FF hens but was not different between the Late-RF (0.0%) and Late-RR (0.0%) hens. Feeding treatment had no effect on LH surge interval within the Early and Late periods, but the interval was shorter (P<0.001) in the Early hens (31.2 hr) than in the Late hens (43.5 hr). Percentage blind LH surges (surges not associated with an oviposition) was not different among groups (7.3% of all surges). The LH baseline concentration was not different among Early (1.69 to 1.87 mg/mL) and Late (1.36 to 1.68 ng/mL) hens. The LH surge amplitude concentration was not different among feeding groups in Early hens and declined in Late hens in comparison to Early hens in each feeding group. The P₄ baseline, P₄ surge amplitude, and overall E₂ concentrations were not different among Early and Late hens within feeding groups. In conclusion, feeding treatment affected abnormal egg production, but had no effect on LH surge interval within the Early and
Late hens, but the mean interval was shorter in Early than Late hens. Also, feeding treatment had no effect on LH surge amplitude, but the mean surge amplitude was much lower in the Late than Early hens.

**INTRODUCTION**

In broiler breeder hens production of settable eggs is improved by feed restriction during growth and reproductive periods in comparison to ad libitum feeding. Broiler breeder hens fed ad libitum during growth are excessively heavy and fat, and have reduced reproductive efficiency (Yu et al., 1992; Eitan et al., 1998; NØddegaard et al., 2000). Broiler breeder hens fed ad libitum during growth lay more abnormal (double yolk, membrane, and soft shelled) eggs in comparison to restricted-fed hens early in the reproductive period (Jaap and Muir, 1968; Hocking et al., 1987; Robinson et al., 1993; Bruggeman et al., 1999). Feed restriction during growth, therefore, is practiced for broiler breeder hens to increase female reproductive efficiency, including increased production of settable eggs, improved fertility and hatchability, and decreased hen mortality (McDaniel et al., 1981; Hocking et al., 1987, 1989; Hocking, 1993).

The onset of sexual maturity in ad libitum-fed and restricted-fed broiler breeder
hens started 2 to 3 wk or 4 to 5 wk later, respectively, when the hens were photostimulated at between 19 to 22 wk of age (Noddegaard, et al., 2000; Liu et al., 2004b). In the management of broiler breeder hens, hens are reared in the floor pens rather than in cages, and housed in floor pens during the reproductive period. Several studies of egg production of broiler breeder hens housed in floor pens have reported that egg production of ad libitum-fed hens started earlier, the peak rate was maintained for a shorter duration, and declined more rapidly in comparison to restricted-fed hens (Hocking et al., 1994; Eitan et al., 1998; Noddegaard et al., 2000). The pattern of egg production of a line (Ross 508) of restricted-fed (RR) broiler breeder hens is characterized by a sharp increase to a peak of about 85% about 4 wk after starting laying, and then a decline by about 1% weekly during 30 wk of production, whereas ad libitum-fed (FF) hens increased to a peak at about 70% 3 wk after starting laying and then declined by about 1.5 % weekly during 20 wk of production. The egg production pattern of ad libitum-fed hens switched from restricted feeding to ad libitum feeding when photostimulated (RF) is between that of FF and RR hens (Figure 4.1).

Direct evidence from in vivo studies has shown that single or multiple mature (F₁) hierarchical follicles were induced to ovulate after acute injection of luteinizing
hormone (LH) in hypophysectomized laying chicken hens, indicating LH induces ovulation (Opel and Nalbandov, 1961a, b). Progesterone (P4) mainly from F1 follicle granulosa cells also surged at the same time as LH, and each P4 surge was coincident with an LH surge (Liu et al., 2004b). Preovulatory P4 surges were maintained for a longer time and have a broader plateau than LH surges in broiler breeder hens (Liu et al., 2001b) and turkeys (Yang et al., 1997; Liu et al., 2001b). The duration of the ascending limb of the LH surge was about one third (1.5 to 2.5 h) and the duration of the descending limb was about two thirds (4.0 to 4.9 h) of the total duration (5.5 to 7.5 h) of the LH surge in both restricted-fed and ad libitum-fed broiler breeder hens (Liu et al., 2004b).

Spontaneous ovulations occur about 6 to 8 h after preovulatory surges of LH and P4 in broiler hens (Liu et al., 2004b), turkeys (Mashaly et al., 1976; Liu et al., 2001c; Bacon et al., 2002), and quail (Doi et al., 1980). A series of studies in turkey hens has shown that the decline in egg production rate during the reproductive period is mainly determined by the interval between preovulatory LH surges (Liu et al., 2001a, c, 2002). We also reported that the poor egg production rate (55%) in heavy weight turkey hens (RBC3 line) was associated with a longer interval between preovulatory LH surges in comparison to light weight turkey hens (Egg line) with good egg production rate (85%)
early in the reproductive period (Liu et al., 2001c). Thus, a longer interval between LH surges was the primary determinant of poor egg production late in comparison to early in the reproductive period in turkey hens (Liu et al., 2002).

Production of estradiol-17β (E_2) was mainly from theca cells of small ovarian follicles in domestic chicken hens (Nitta et al., 1991a, b) and turkeys (Porter et al., 1989). Production of yolk precursor lipoprotein by liver and development of the oviduct are induced by injection of E_2 in laying hens (Walzem, 1996; Walzem et al., 1999). Peripheral concentrations of E_2 are relatively stable during ovulatory cycles and are independent from preovulatory LH surges in laying turkeys (Liu et al., 2001a, c, 2002) and broiler chickens (Liu et al., 2004b).

The current study was designed to examine: 1) whether higher settable egg production in restricted-fed (RR) broiler breeder hens in comparison to ad libitum-fed (RF and FF) hens was mainly associated with the interval between preovulatory LH surges or characteristics of LH and P_4 surges, and 2) whether the decline in egg production rate with increasing duration of the reproductive period was associated with differences in the interval between preovulatory surges of LH or characteristics of LH and P_4 surges. It was hypothesized that feed restriction did not alter the frequency of
LH surges, but that the frequency of LH surges declined with progression of the reproductive period.

MATERIALS AND METHODS

Birds and Blood Sample Collection

Broiler breeder hens (Ross 508) were raised in the floor pens with continuous lighting (24L:0D) and with ad libitum access to feed until 1 wk of age. The hens were separated into 2 duration of reproduction groups and 3 feeding program groups, based on different feeding conditions (Table 4.1). Hens were sampled early (Early) at the peak of egg production and late (Late) after egg production declined approximately 25%. The first group of hens was ad libitum fed during the growth and reproductive periods (FF). The second group of hens was restricted-fed during growth and then switched to ad libitum feeding at 22 wk of age when photostimulated (RF). The third group of hens was restricted fed during both growth and reproductive periods (RR). The program of feed restriction used in the second and third groups was based on recommendations of the supplier (Aviagen, 2000). All hens were given a 8L:16D photoperiod from 1 to 22 wk of age. All hens were housed in cages (32X42X46 cm) with slatted rubber floor mats
and photostimulated with 16L:8D to induce sexual maturity at 22 wk of age. All hens were switched to a 24L:0D photoperiod to allow LH surges to free run immediately following cannulation and 3 to 5 d before serial blood sampling began. The cannulation procedure and success rate of complete sample collection (hourly for 240 h) for the hens was described in detail in Chapter 2 (Liu et al., 2004a). The blood samples were taken hourly (1.5 mL) for 240 h early (Early, 3 to 6 wk of production) in the reproductive period and after production had declined by about 25% (Late, 20 to 30 wk of production). The hens used in each trial met the following reproduction requirement: before cannulation, all hens laid eggs for the previous 2 wk. Egg production of individual hens was recorded daily. The hens were palpated 3 times a day to determine the presence of an egg in the uterus (soft or hard shelled) and to identify the specific association between preovulatory LH surges, ovulation, and oviposition.

**LH, P₄, and E₂ Assays**

Concentrations of LH were measured by radioimmunoassay (RIA) as previously described using 100 µL plasma for each duplicate (Proudman et al., 1984; Bacon and Long, 1996). All blood samples collected every h for 240 h were assayed. The
intrassay and interassay coefficients of variation (CV) of plasma pools from laying broiler breeder hens with concentrations of LH (3.10±0.21 ng/mL) were 7.3 % and 6.9 %, respectively.

The concentration of P₄ was measured by RIA using 12 µL of plasma for each duplicate (Yang et al., 1997). Samples analyzed for P₄ were selected retrospectively in relation to surges of LH. Samples taken at 5 and 6 h before the peak of LH surges were assayed to estimate baseline concentrations of P₄. Samples taken 2 and 3 h after the peak of LH surges were assayed to estimate P₄ surge amplitude concentrations (Liu et al., 2004b). The intraassay and interassay CV of a plasma pool (3.51±0.27 ng/mL) from laying broiler breeder hens were 9.4 % and 7.7 %, respectively. The mean percentage recovery after extraction was 86.8%, determined by adding ³H-P₄ to control plasma. All plasma samples were corrected for percentage recovery.

The concentration of E₂ was determined by RIA using 100 µL plasma for each duplicate for samples collected every 12 h by the method of Chen et al. (1999). The intraassay and interassay CV of a pool of plasma with E₂ concentrations (mean=0.424±0.41 ng/ml) were 9.1 % and 9.6%, respectively. The percentage recovery after extraction was 89.5%. All plasma samples were corrected for percentage recovery.
Statistical Analyses

The LH raw data were evaluated by the Pulsar algorithm to identify LH and P₄ preovulatory surges, and to calculate baseline, overall, and peak amplitude concentrations, and preovulatory surge durations of LH (Merriam and Wachter, 1982). For LH, the G-values used for Pulsar analysis were G(1)=50, G(2)=2.6, G(3)=1.9, G(4)=1.5, G(5)=1.2. The standard deviations for LH used for PULSAR analyses was (7.27X)/100, where X is the sample concentration measured in an individual sample. The statistical model for data analyses was a 2X3 factorial with interaction. The main effects were duration of egg production (Early and Late) and feeding program (FF, RF, and RR). Individual hen was error term for all statistical analyses. The following measurements were analyzed: 1) body weight after serial bleeding; 2) number of total eggs laid the 10 d before serial bleeding; 3) number of total eggs laid the 10 d during serial bleeding; 4) number of settable eggs laid the 10 d during serial bleeding; 5) number of abnormal of eggs laid the 10 d during serial bleeding; 6) overall mean concentrations of E₂; 7) baseline concentrations of LH and P₄; 8) peak amplitude concentrations of LH and P₄; 9) interval between preovulatory surges of LH; and 10) percentage of LH blind surges. In
addition, Pearson correlations coefficients were calculated among all measurements.

The MINITAB\textsuperscript{11} software program was used for all statistical analyses.

RESULTS

Body weight was heavier in the Late than in the Early hens, and was different among feeding programs (FF>RF>RR, Table 4.2). No interaction between duration of reproduction and feeding program was detected for body weight (Table 4.3). Total egg production the 10 d before cannulation was higher in the Early than Late hens, and higher in the RR than FF hens. There was no interaction between duration of reproduction and feeding program for this or other egg production traits (Table 4.3). Total egg production the 10 d during serial bleeding was higher in the Early than Late hens but not different among feeding programs. Total egg production within each duration of production-feeding program group was not different the 10 d before cannulation and 10 d during serial bleeding (Table 4.2). Settable egg production the 10 d during serial bleeding was higher in the Early than Late hens, and in the RR than FF feeding program hens. Abnormal egg production the 10 d during serial bleeding was higher in the Early than Late hens (P=0.09) and in the FF than RR feeding program hens (P=0.07).

\textsuperscript{1} MINITAB, State College, PA 16801-3008
Patterns of free running preovulatory LH and P₄ surges and E₂ concentrations of representative hens over the 10 d of serial bleeding are shown in Figure 4.2. The total numbers of preovulatory LH surges over 10 d of serial bleeding for the Early-FF, Early-RF, and Early-RR, Late-FF, Late-RF, and Late-RR were 70, 81, 102, 64, 72, and 65. The patterns of hormones for the hens with the shortest intervals between LH surges within their group are presented in the top panels, for the hens with intervals between LH surges closest to the mean within their group in the middle panels, and for the hens with the longest intervals between LH surges within their group in the bottom panels of Figure 4.2 a to f. Surges of LH and P₄, not associated with oviposition of an egg are defined as blind surges (i.e. Early-FF hen #5; Figure 4.2a). The Late-FF hen #66 had 3 blind surges in a row over 10 d of serial bleeding (Figure 4.2d). This hen also held a hard shelled egg in the uterus for over 2 d, and the oviposition time was independent from the time of LH surges. The Late-FF hen #72 laid 4 soft shelled eggs over the 10 d period, with 2 soft shelled eggs laid in 1 h immediately following the oviposition of an extra-calcified egg (Figure 4.2d). The patterns of the LH surges and P₄ peak concentration associated with these 2 soft shelled eggs in the Late-FF hen #72 (Figure 4.2d) were not different from surges associated with settable eggs oviposited by this hen.
At necropsy, one hard shelled egg (39.52 g) and 1 soft shelled egg (3.34 g) were found in the body cavity of the Late-FF hen #66 (Figure 4.2d). This hen was fed fat soluble dyes for 2 wk before cannulation (Chapter 6), but these 2 eggs did not have dye deposited in the yolks, suggesting the eggs had been in the body cavity for at least 4 wk. The Late-RF hen #86 (Figure 4.2e) had the longest interval between LH surges (mean=85.5 h) over 10 d of serial bleeding and this hen held a hard shelled egg in her uterus for over 3 d based on palpation and egg production records.

The peripheral pattern of E$_2$ concentrations was stable and without surges associated with LH surges and did not have a rhythm during ovulatory cycles. The E$_2$ concentrations were independent from preovulatory LH surges in all groups of broiler breeder hens (Figure 4.2 a to f).

Baseline concentrations of LH were higher in the Early than Late hens, but were not different among feeding programs (Table 4.4). There was no interaction between duration of reproduction and feeding program for LH baseline concentration or other hormone characteristics (Table 4.5). Surge amplitude concentration of LH was higher in the Early than Late hens and in the FF than RR hens. The LH surge interval was longer in the Late hens than Early hens, but not different among feeding programs. The
percentage of LH blind surges was not affected by duration of reproductive period or feeding program. The baseline concentration of \( P_4 \) was higher in the Early hens than in the Late hens, but not different among feeding programs. This difference was due to the Early RF hens having a higher \( P_4 \) baseline concentration than the Late-FF hens (Table 4.5). The \( P_4 \) surge amplitude concentration and concentration of \( E_2 \) were not different between Early and Late hens or among feeding treatments.

Distributions of all LH surge intervals are shown in Figure 4.3. Intervals between 20 h and 50 h were bimodally distributed, with 74% of intervals between 20 and 32 h and 26% between 36 and 48 h for the Early hens (Figure 4.3a), and 41% of intervals between 24 and 32 h and 59% between 36 and 52 h for the Late hens (Figure 4.3b). Hen mean surge intervals are shown in Figure 4.4. Hen mean LH surge interval was not as widely dispersed in the Early hens (Figure 4.4a) as in the Late hens (Figure 4.4b).

Estimated egg production, calculated by \( \frac{24 \, \text{h}}{\text{LH surge interval}} \), was similar to the total egg production rate in each feeding group (Table 4.5). The estimated egg production rate would be lower than actual total egg production rate if percentage blind LH surges which was not different between Early and Late hens or among feeding programs was included in the calculations.
The LH baseline concentration was negatively associated with duration of reproduction but positively associated with LH amplitude and P₄ baseline concentrations (Table 4.6). The LH amplitude concentration was negatively associated with duration of reproduction and LH surge interval, and positively associated with LH baseline and E₂ concentrations. The LH surge interval was positively associated with duration of reproduction and negatively associated with LH amplitude and P₄ baseline concentrations. Percentage of blind LH surges was not associated with any measurement of LH, P₄, and E₂. The P₄ baseline concentration was positively associated with LH baseline concentration and negatively associated with LH surge interval. The P₄ amplitude concentration was positively associated only with P₄ baseline concentration among all hormonal measurements. Concentration of E₂ was positively associated only with LH amplitude concentration.

**DISCUSSION**

The pattern of egg production, calculated based on individual hen egg production (Leghorn hens: Grossman et al., 2000; Turkey hens: Liu et al., 2001c; Broiler breeder hens: current study) is shown in Figure 4.5. The patterns are different from the
studies where egg production rate was calculated based on the floor pen housing and first egg within the pen as starting point for duration of egg production (Noddegaard et al., 2000). From Figure 4.5, it is apparent that the initial egg production rate is near the peak rate, then declines after a period of peak production that is positively related to duration of peak production. Also, the data in Figure 4.5 include all abnormal eggs since all hens were housed in cages (chickens) or trap-nested (turkeys). Many abnormal eggs may be broken and lost under floor housing conditions when hens are not trap-nested.

In turkeys, a longer interval between preovulatory LH surges is associated with a poorer egg production rate (peak at 55%) in a sire line (RBC3) in comparison to a line (Egg) with excellent egg production rate (peak at 85%). In turkeys, the interval between LH surges and surge amplitude concentration both decline late in the reproductive period as the rate of egg production declines (Liu et al., 2001c). The current study in broiler breeder hens was designed to induce different egg production rates by various feeding programs during growth and reproduction periods, and at different durations of egg production. Thus, unlike the turkey hen studies, only a single genetic background was used in the current study.
Settable egg production is increased by restricted feeding in broiler breeder hens (Yu et al., 1992; Noddegaard et al., 2000). The current study shows that RR hens laid more settable eggs (P>0.05) than FF hens and FF hens laid more abnormal eggs (P=0.07) in comparison to RR hens. Also, total (settable plus abnormal) eggs were not different among feeding treatments, suggesting that FF hens lay more abnormal eggs in response to ad libitum feeding, resulting in lower settable egg production. The Early hens had higher total and settable egg production than the Late hens among feeding treatments, indicating duration of reproduction is an important factor affecting the decline in production total and settable eggs. Feeding program affected settable egg production, probably by decreasing the number of abnormal eggs produced by hens restricted fed during growth and reproductive periods.

Oviposition was usually associated with preovulatory surges during ovulatory cycles. Of 446 total eggs, 33 were abnormal eggs in this study. Patterns of preovulatory LH surges and P₄ surge concentrations associated with abnormal (soft shelled and double yolked) eggs were not very different from those of settable eggs. Some hens (Figure 4.2a and d) oviposited 2 soft shelled eggs in 1 h, but the surge patterns of LH and P₄ surge concentration were not different from settable eggs laid by each hen.
This indicates that one preovulatory LH surge can induce ovulation of ova from 2 mature hierarchical follicles, that these ova entered the oviduct and resulted in oviposition of 2 soft-shelled eggs, which were oviposited earlier than expected. No unusual hormonal patterns or concentrations were associated with the production of these abnormal unsettable eggs.

The interval between preovulatory LH surges accounts for the difference in egg production in turkey hens early and late in the reproductive period, and in lines with different egg production rates (Liu et al., 2001c, 2002). The current data show that the surge interval was not different between feeding treatments either early or late in the reproductive period within a single strain of broiler breeder hens. This suggests that laying more abnormal eggs by the Early-FF and Late-FF hens in comparison to Early-RR and Late-RR hens was not due to a difference in the interval between preovulatory LH surges.

A 24L:0D photoschedule was used in this study so that LH surges would free run. In the study, several hundred LH and P₄ surges were detected and all P₄ surges are coupled to LH surges, but not all LH and P₄ surges were associated with ovipositions (Liu et al., 2004b; current study). Under a diurnal photoschedule, subsequent
ovipositions occur later than previous ones in a clutch in laying chickens (Etches, 1996), quail (Opel, 1966), and turkeys (Woodard et al., 1963; Liu et al., 2001a, c, 2002; Bacon et al., 2002). The free running rhythm of preovulatory LH surges (>24 h) was similar to the rhythm under a diurnal photoschedule in that subsequent LH surges occurred later than previous ones, probably during open periods. The next LH surge would wait approximately an additional d (pause d) and occur early in the open period in the next clutch. A long interval between LH surges was mainly associated with poorer egg production in turkeys (Liu et al., 2001, 2002). The free running rhythm of LH surges in Late laying hens shows that poorer egg production rate of the Late hens is induced by both a longer intra clutch surge interval and by more pause d. In the current study, however, it is not known if the factors that determine the rhythm of LH surges come from the ovary, hypothalamus, or anterior pituitary, or if they change with increasing duration of the reproductive period.

With the hens photostimulated with a 24L:0D photoperiod, the distribution of all LH surge intervals was arbitrary separated into short [26.5 (74%) and 28.4 (41%) hr] and long [39.3 (26%) and 43.7 (59%) hr] interval groups in Early and Late hens, respectively. The short intervals probably represent intra clutch intervals, and the longer
interval pauses. A wider distribution of all LH surge intervals was found in Late hens in comparison to Early hens and some surge intervals were longer than 96 hr, suggesting free running LH surges had a larger variation late in the reproductive period. A higher percentage (74%) of short duration LH surge intervals was found in Early hens and a higher percentage (59%) of long duration surge interval was found in Late hens, suggesting more pause days were associated with poorer egg production late in the reproductive period.

The percentage of LH blind surges, surges not retrospectively associated with an oviposition, was not an important factor in the decline in egg production rate late in the reproductive period or differences in egg production among feeding programs. Possible explanation for the blind LH surge is internal ovulation which occurs when an ovum is ovulated but does not enter the infundubulum so a complete egg cannot be formed (Liu et al., 2001c; Bacon et al., 2002). A higher incidence of internal ovulation has been reported early in the reproductive period in the turkey hens selected for rapid growth rate in comparison to the turkey hens selected for increased egg production rate (Melnychuk et al., 1997; Liu et al., 2001c). The current study in broiler breeder hens, however, does not show an effect of feeding program on incidence of blind LH surges, suggesting that
ad libitum feeding did not contribute to a higher rate of blind LH surges, and thus lower egg production.

A higher baseline concentration of LH was associated with initiation of egg production and declined with increasing duration of the reproductive period in turkey hens (Chapman et al., 1994; Guemene and Williams, 1994; Liu et al., 2002). The baseline LH concentration decreased with duration of the reproductive period, suggesting baseline concentration might be an important factor associated with egg production rate in broiler breeder hens. The current study shows that LH baseline concentration was positively associated with LH surge amplitude concentration. Confounding factors between hen age and duration of the reproductive period were separated in turkey hens showing that LH baseline and surge amplitude concentrations were dependent on duration of the reproduction period rather than hen age (Liu et al., 2001a). The LH surge amplitude concentrations also declined late in the reproductive period in turkey hens. The cause of these declines may be due to older hens secreting less LHRH, being less sensitive to LH releasing hormone (LHRH) or some other factor possibly associated with LH isoform changes (Etches et al., 1983; King et al., 1986). To the author’s, a cannulation technique for serial collection of blood samples from the eminence medium
to measure LHRH secretion has not been developed for any avian species.

Progesterone is mainly secreted from granulosa cells of large yolky follicles in birds (Bahr et al., 1983; Porter et al., 1989). Direct evidence shows that higher $P_4$ baseline concentrations induced by chronic injection of progesterone is associated with decreased egg production in quail (Liu and Bacon, 2004a) and broiler breeder hens (Liu and Bacon, 2004b), and development of polycystic ovarian syndrome in young turkey hens (Bacon and Liu, 2004). Early-FF and Early-RF hens had more hierarchical follicles than Early-RR and Late-RR hens but the increase in yolky follicles did not contribute to higher $P_4$ baseline and $P_4$ surge amplitude concentrations, in agreement with turkey studies (Liu et al., 2001c). Since no increase in $P_4$ baseline or surge amplitude occurred with increasing duration of the reproductive period, $P_4$ is probably not the cause of decreased egg production late in the reproductive period, unless hens late in the reproductive period are more sensitive to $P_4$.

Estradiol-17β is an important factor to stimulate yolk precursor lipoprotein secretion by the liver during egg production in birds (Walzem, 1996; Walzem et al., 1999). Several studies in turkeys (Liu et al., 2001c, 2002; Bacon et al., 2002) and in broiler breeder chickens (Liu et al., 2004a; current study) have shown that $E_2$ concentrations
only slightly changed during ovulatory cycles and were mostly independent from preovulatory LH surges, suggesting E₂ does not play an important role in altering the frequency of preovulatory LH surges, ovulation rate, and egg production rate. Overall mean E₂ concentration was positively associated with LH surge amplitude concentration but not with LH baseline concentration and duration of reproduction. This suggests that LH amplitude concentration may be a signal for decreased yolk precursor lipoprotein secretion by the liver late in the reproductive period in broiler breeder hens.

In summary, production of total eggs was not affected by feeding treatments early or late in the reproductive period. Early-FF and Late-FF hens laid more abnormal (double yolked, soft shelled, and thin shelled) eggs than Early-RR and Late-RR hens, respectively. Feeding program had no effect on LH surge interval within the Early and Late hens, but the mean hen interval was shorter in the Early hens (31.2 h) than in the Late hens (43.7 h). Percentage blind LH surges was a very minor modification on the difference in egg production among feeding treatments and ages. The LH baseline and LH surge amplitude concentrations were not altered by feeding treatments early and late in the reproductive period but LH surge amplitude concentration declined with advancing duration of the reproductive period. The P₄ baseline and surge amplitude and overall E₂
concentrations were not changed throughout the reproductive period or by feeding program.

REFERENCES


<table>
<thead>
<tr>
<th>Variable</th>
<th>Early-FF</th>
<th>Eearly-RF</th>
<th>Early-RR</th>
<th>Late-FF</th>
<th>Late-RF</th>
<th>Late-RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hen (n)</td>
<td>10</td>
<td>10</td>
<td>13</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Age when sampled (wk)</td>
<td>25-26</td>
<td>29-30</td>
<td>32-33</td>
<td>43-44</td>
<td>49-50</td>
<td>58-59</td>
</tr>
<tr>
<td>Duration of production(^1) (wk)</td>
<td>4-5</td>
<td>3-4</td>
<td>5-6</td>
<td>19-20</td>
<td>22-23</td>
<td>30-31</td>
</tr>
<tr>
<td>Feeding(^2) (1-22 wk)</td>
<td>F</td>
<td>R</td>
<td>R</td>
<td>F</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Feeding (23 wk- end trial)</td>
<td>F</td>
<td>F</td>
<td>R</td>
<td>F</td>
<td>F</td>
<td>R</td>
</tr>
</tbody>
</table>

\(^1\) The hens were photostimulated with 16L:8D at 22 wk of age and switched to 24L:0D to allow luteinizing hormone (LH) surges to free run when cannulated.

\(^2\) All hens were ad libitum-fed before 1 wk of age. The hens were restricted-fed (R) based on the recommendation of the supplier (ROSS 508 broiler breeder hens) or ad libitum-fed (F).

Table 4.1. Hen number, age, duration of production, and feeding treatments for the 6 trials.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Duration of the reproductive period</th>
<th>Feeding program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>4.35±0.08(^{b})</td>
<td>4.93±0.07(^{a})</td>
</tr>
<tr>
<td>Total eggs 10 d before(^{1}) (n)</td>
<td>8.15±0.25(^{a})</td>
<td>5.76±0.23(^{b})</td>
</tr>
<tr>
<td>Total eggs 10 d during(^{2}) (n)</td>
<td>7.79±0.29(^{a})</td>
<td>5.35±0.27(^{b})</td>
</tr>
<tr>
<td>Settable eggs 10 d during (n)</td>
<td>7.12±0.30(^{a})</td>
<td>5.07±0.28(^{b})</td>
</tr>
<tr>
<td>Abnormal eggs 10 d during (n)</td>
<td>0.67±0.17</td>
<td>0.28±0.16</td>
</tr>
</tbody>
</table>

\(^{a-c}\) Means with different superscript letters within rows and main effect are different \((P<0.05)\)

1 Total eggs [including abnormal (soft, thin shelled, and double yolked eggs) and settable eggs] laid the 10 d before cannulation.

2 Total eggs laid the 10 d during hourly blood sampling for 240 h.

Table 4.2. Main effect means±SE for body weight, total eggs laid the 10 d before cannulation and during the 10 d hourly blood sampling, and settable and abnormal egg production during the 10 d of hourly blood sampling.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Early-FF</th>
<th>Early-RF</th>
<th>Early-RR</th>
<th>Late-FF</th>
<th>Late-RF</th>
<th>Late-RR</th>
<th>( P = )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>5.1±0.1</td>
<td>4.2±0.1</td>
<td>3.7±0.1</td>
<td>5.5±0.1</td>
<td>4.8±0.1</td>
<td>4.6±0.1</td>
<td>0.16</td>
</tr>
<tr>
<td>Total eggs 10 d before(^1)</td>
<td>7.10±0.43</td>
<td>8.70±0.43</td>
<td>8.64±0.41</td>
<td>5.50±0.39</td>
<td>5.54±0.37</td>
<td>6.18±0.41</td>
<td>0.21</td>
</tr>
<tr>
<td>Total eggs 10 d during(^2)</td>
<td>7.40±0.50</td>
<td>8.40±0.50</td>
<td>7.64±0.48</td>
<td>5.33±0.46</td>
<td>5.15±0.44</td>
<td>5.64±0.48</td>
<td>0.30</td>
</tr>
<tr>
<td>Settable eggs 10 d during</td>
<td>6.20±0.51</td>
<td>7.40±0.51</td>
<td>7.64±0.49</td>
<td>4.50±0.47</td>
<td>5.15±0.45</td>
<td>5.64±0.49</td>
<td>0.85</td>
</tr>
<tr>
<td>Abnormal eggs 10 d during</td>
<td>1.20±0.30</td>
<td>1.00±0.30</td>
<td>0.00±0.28</td>
<td>0.83±0.27</td>
<td>0.00±0.26</td>
<td>0.00±0.28</td>
<td>0.17</td>
</tr>
</tbody>
</table>

\(^1\) Total eggs [including abnormal (soft, thin shelled, and double yolked eggs) and settable eggs] laid the 10 d before cannulation.

\(^2\) Total eggs laid the 10 d during hourly blood sampling for 240 h.

Table 4.3. Interaction means±SE for body weight, total eggs laid the 10 d before cannulation and during the 10 d hourly blood sampling, and settable and abnormal egg production during the 10 d of hourly blood sampling.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Duration of the reproductive period</th>
<th>Feeding program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>LH baseline (ng/mL)</td>
<td>1.77±0.07$^a$</td>
<td>1.52±0.07$^b$</td>
</tr>
<tr>
<td>LH surge amplitude (ng/mL)</td>
<td>2.84±0.10$^a$</td>
<td>1.77±0.09$^b$</td>
</tr>
<tr>
<td>LH surge interval (hr)</td>
<td>31.19±1.60$^b$</td>
<td>43.48±1.52$^a$</td>
</tr>
<tr>
<td>LH blind surges$^1$ (%)</td>
<td>4.69±2.27</td>
<td>9.66±2.10</td>
</tr>
<tr>
<td>P₄ baseline (ng/mL)</td>
<td>1.17±0.05$^a$</td>
<td>1.04±0.05$^b$</td>
</tr>
<tr>
<td>P₄ surge amplitude (ng/mL)</td>
<td>3.78±0.13</td>
<td>3.77±0.12</td>
</tr>
<tr>
<td>E₂ (ng/mL)</td>
<td>0.24±0.01</td>
<td>0.23±0.01</td>
</tr>
</tbody>
</table>

$^a,b$ Means with different superscript letters within rows and main effect are different ($P \leq 0.05$)

$^1$ LH blind surges are LH surges not retrospectively associated with ovipositions.

Table 4.4. Main effect means±SE for luteinizing hormone (LH) concentration, LH baseline, and surge amplitude concentrations, LH surge interval, percentage LH blind surges, progesterone (P₄) baseline and surge amplitude concentrations, and estradiol-17β concentration (E₂) in broiler breeder hens early and late in the reproductive period and among feeding programs.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Early-FF</th>
<th>Early-RF</th>
<th>Early-RR</th>
<th>Late-FF</th>
<th>Late-RF</th>
<th>Late-RR</th>
<th>( P = )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH baseline (ng/mL)</td>
<td>1.69±0.13</td>
<td>1.87±0.12</td>
<td>1.78±0.11</td>
<td>1.68±0.11</td>
<td>1.53±0.11</td>
<td>1.36±0.11</td>
<td>0.35</td>
</tr>
<tr>
<td>LH surge amplitude (ng/mL)</td>
<td>3.05±0.17</td>
<td>2.79±0.16</td>
<td>2.68±0.15</td>
<td>2.13±0.16</td>
<td>1.81±0.14</td>
<td>1.37±0.15</td>
<td>0.53</td>
</tr>
<tr>
<td>LH surge interval (hr)</td>
<td>32.4±3.1</td>
<td>30.3±2.9</td>
<td>31.0±2.7</td>
<td>40.8±2.8</td>
<td>47.3±2.56</td>
<td>42.4±2.7</td>
<td>0.29</td>
</tr>
<tr>
<td>LH blind surges(^1) (%)</td>
<td>9.9±4.1</td>
<td>2.9±3.9</td>
<td>3.0±3.6</td>
<td>13.2±3.7</td>
<td>9.3±3.4</td>
<td>5.4±3.6</td>
<td>0.97</td>
</tr>
<tr>
<td>( P_4 ) baseline (ng/mL)</td>
<td>1.19±0.09</td>
<td>1.29±0.09</td>
<td>1.04±0.08</td>
<td>0.89±0.08</td>
<td>1.12±0.08</td>
<td>1.12±0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>( P_4 ) surge amplitude (ng/mL)</td>
<td>4.15±0.23</td>
<td>3.83±0.23</td>
<td>3.36±0.21</td>
<td>3.71±0.21</td>
<td>3.81±0.20</td>
<td>3.77±0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>( E_2 ) (ng/mL)</td>
<td>0.23±0.02</td>
<td>0.24±0.02</td>
<td>0.25±0.01</td>
<td>0.25±0.02</td>
<td>0.24±0.01</td>
<td>0.21±0.01</td>
<td>0.30</td>
</tr>
<tr>
<td>Estimated egg production rate(^2) (%)</td>
<td>74.5</td>
<td>79.3</td>
<td>77.2</td>
<td>58.9</td>
<td>50.7</td>
<td>56.6</td>
<td>--</td>
</tr>
</tbody>
</table>

\(^1\) LH blind surges are LH surges not retrospectively associated with ovipositions.

\(^2\) Egg production rate estimated by 24 h ÷ mean LH surge interval.

Table 4.5. Interaction means±SE for luteinizing hormone (LH) concentration, LH baseline and surge amplitude concentrations, LH surge interval, percentage blind LH surges, progesterone (\( P_4 \)) baseline and surge amplitude concentrations, estradiol-17\( \beta \) concentration (\( E_2 \)), and estimated egg production rate of broiler breeder hens early and late in the reproductive period and among feeding programs.
Table 4.6. Pearson correlation coefficients (r) and statistical probability of significance (P) among duration of reproduction, LH baseline and LH amplitude (amp.) concentrations, interval between LH surges, percentage LH blind surges, P₄ baseline and P₄ amplitude concentrations, and overall mean E₂ concentrations in broiler breeder hens.

<table>
<thead>
<tr>
<th></th>
<th>Duration</th>
<th>LH baseline</th>
<th>LH amp.</th>
<th>LH interval</th>
<th>Blind surge (%)</th>
<th>P₄ baseline</th>
<th>P₄ Amp</th>
<th>E₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH baseline</td>
<td>r=-0.39</td>
<td>r=-0.74</td>
<td>r=0.54</td>
<td>r=-0.11</td>
<td>r=-0.15</td>
<td>r=0.03</td>
<td>r=0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.00</td>
<td>P=0.00</td>
<td>P=0.00</td>
<td>P=0.38</td>
<td>P=0.22</td>
<td>P=0.83</td>
<td>P=0.25</td>
<td></td>
</tr>
<tr>
<td>LH amp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r=0.30</td>
<td>r=-0.17</td>
<td>r=-0.20</td>
<td>r=0.27</td>
<td>r=-0.06</td>
<td>r=-0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.01</td>
<td>P=0.17</td>
<td>P=0.10</td>
<td>P=0.03</td>
<td>P=0.65</td>
<td>P=0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r=0.38</td>
<td>r=0.10</td>
<td>r=0.03</td>
<td>r=0.19</td>
<td>r=0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.00</td>
<td>P=0.43</td>
<td>P=0.81</td>
<td>P=0.12</td>
<td>P=0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blind surge (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r=0.04</td>
<td>r=-0.24</td>
<td>r=0.08</td>
<td>r=0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.73</td>
<td>P=0.05</td>
<td>P=0.51</td>
<td>P=0.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P₄ baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r=-0.13</td>
<td>r=0.04</td>
<td>r=-0.02</td>
<td>r=0.29</td>
<td>r=0.74</td>
<td>r=0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.74</td>
<td>P=0.03</td>
<td>P=0.22</td>
<td>P=0.18</td>
<td></td>
<td>P=0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P₄ Amp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r=0.26</td>
<td>r=0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.03</td>
<td>P=0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>r=0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig 4.1. Hen $d^{-1}$ % egg production rate of ad libitum-fed (FF), ad libitum-fed switched from restricted-fed (RF), and restricted-fed (RR) broiler breeder hens. The hens were photostimulated with 16L:8D at 22 wk of age and were switched to 24L:0D when cannulated to allow LH surges to free run. Weeks of production were calculated for each hen from day of first egg. Circles represent the times of serial blood sampling Early and Late in the reproductive period.
Fig 4.2. Hourly plasma concentrations of luteinizing hormone (LH) for 240 h in representative broiler breeder hens [data presented are for hens with shortest (top), closest to mean (middle), and longest (bottom) mean surge interval of each experimental group]. Closed circles at the top of the panels represent the approximate times of oviposition of eggs based on palpation and egg collection records. Open circles at top right of some panels represent oviposition of soft shell eggs (Early FF#5) or of a soft shelled egg in the uterus (SS) at necropsy. Arrows give assigned association of an LH surge to the oviposition of an egg based on palpation and egg collection records. Surges not associated with ovipositions are called blind LH surges. The question mark(s) in some panels indicate blind LH surges.
Fig 4.2: Continued

A

Early FF #12

Early FF #5

Early FF #16

A (Continued)
Fig 4.2: Continued

B

Early RF #40

Early RF #26

Early RF #32

B (Continued)
Fig 4.2: Continued

Early RR #45

Early RR #47

Early RR #60

C (Continued)
Late FF #62

Late FF #66

Late FF #72

D (Continued)
Fig 4.2: Continued

Late RF #68

Late RF #85

Late RF #86

E (Continued)

157
Fig 4.2: Continued

Late RR #127

Late RR #128

Late RR #126

F (Continued)
Fig 4.3. Distributions of all LH surge intervals of FF, RF, and RR hens early (a) or late (b) in the reproductive period.
Fig 4.4. Distributions of hen mean LH surge intervals of FF, RF, and RR hens early (a) or late (b) in the reproductive period.
Fig 4.5. Egg production pattern in 1) ad libitum-fed (FF), ad libitum-fed switched from restricted-fed (RF), and restricted-fed (RR) broiler breeder hens (current study).  2) Egg line turkey hens with excellent egg production rate and RBC line turkey hens with poorer egg production rate (Liu et al., 2001c).  3) Leghorn laying hens (Grossman et al., 2000). Circles represent the times of serial blood sampling early and late in the reproductive period.
CHAPTER 5

PERIPHERAL CONCENTRATIONS OF LEPTIN AND INSULIN WERE NOT

CHANGED WITH DURATION OF THE REPRODUCTIVE PERIOD BUT

INSULIN CONCENTRATION LOWERED IN AD LIBITUM-FED BROILER

BREEDER HENS

ABSTRACT

Production of settable eggs is increased by feed restriction of broiler breeder hens whereas ad libitum-fed hens become excessively fat and have the reproductive disadvantage of laying more unsettable abnormal eggs (double yolked, soft shelled, thin shelled, and extracalcified). Leptin is a hormone mainly secreted by adipose tissue and liver, and is though to be positively associated with adiposity. Insulin is a hormone secreted by the pancreas and its release is mainly associated with meal time. It was hypothesized that leptin concentration would be higher in ad libitum-fed hens with heavier fat pads in comparison to restricted-fed broiler breeder hens with reduced fat pads,
and insulin concentrations would be increased after feeding in restricted-fed hens. Three groups of broiler breeder hens were used to test these hypotheses early (Early, at peak of production) and late (Late, when egg production rate had declined 25%) in the reproductive period. The first group of hens was ad libitum-fed during both growth and reproductive periods (FF). The second group of hens was restricted-fed during the growth period and then switched to ad libitum feeding during the reproductive period (RF). The third group of hens was restricted-fed during both growth and reproductive periods (RR). Blood samples were collected hourly for 240 h early (Early, at 3 and 6 wk of egg production) and late (Late, 19 to 31 wk of production) in the reproductive period. The fat pad weight was heavier in the Late hens than in the Early hens and in the FF hens than in the RF and RR hens. The interval between luteinizing hormone (LH) surges was longer in the Late hens than in the Early hens but not different among feeding treatments. Leptin and insulin concentrations were not different between the Early and Late hens. Leptin concentrations were not different among feeding programs but insulin concentrations were higher in the RR and RF hens than in the FF hens and were generally increased after meal feeding. Concentrations of leptin and insulin were not altered by preovulatory LH surges during 10 d of sampling. It was concluded that leptin and
insulin concentrations were not associated with differences in body weight, fat pad weight, interval between LH surges, and total egg production during either the early or late portions of the reproductive period in broiler breeder hens.

**INTRODUCTION**

Leptin, a product of the obese (ob) gene, a 16 KD polypeptide hormone, is primarily produced by adipose tissue, liver, embryonic liver and yolk sac in chickens (Taouis et al., 1998; Ashwell et al., 1999; Friedman-Einat et al., 1999). Leptin plays an important role in the regulation of feed intake (appetite) and energy expenditure to alter growth rate (Ashwell et al., 2001; Richards, 2003; Sahu, 2003; Bjorbaek and Kahn, 2004) and is involved in reproduction via stimulation of secretion of gonadotropins (Barash et al., 1996; Chehab et al., 1996). Direct evidence has shown that intracerebroventricular, intrahypothalamic, intraperitoneal, or intramuscular injections of recombinant mouse or chicken leptin decreased food intake in chickens (Denbow et al., 2000; Dridi et al., 2000; Taouis et al., 2001), great tits (Lohmus et al., 2003), mice (Chen et al., 1996), rats (Cusin et al., 1996), and pigs (Barb et al., 1998).

It has been reported that the concentration of leptin is positively associated with
body fat in rodents (Maffei et al., 1995) and sheep (Blache et al., 2000; Delavaud et al., 2000), and has a diurnal pattern associated with feeding pattern that peaked at night (rat: Saladin et al., 1995; mice: Ahren, 2000; Human: Langendonk et al., 1998; Licinio et al., 1998). Leptin concentrations are increased in the fed state and decreased in the fasted state in humans (Boden et al., 1996; Kolaczynski et al., 1996) and sheep (Kumar et al., 1998; Marie et al., 2001). In broiler breeder hens, leptin mRNA expression increased from 1 to 6 wk of age and was highly correlated with metabolic body weight when fat accumulation was limited, but there was no association between 6 and 12 wk of age, when fat accumulation had increased (March et al., 1984; Ashwell et al., 2001). The pattern of leptin secretion in broiler breeder hens was similar to that of mammals in that leptin secretion was highly associated with adiposity and increased with age until puberty (Cheung et al., 1997; Mantzoros et al., 1997; Gruaz et al., 1998).

The onset of puberty was advanced when immature animals were administrated leptin (Ahima et al., 1997; Chenab et al., 1997). In young women sampled every 7 min for 24 h during d 8 and 11 of the menstrual cycle, the pattern of leptin showed an ultradian pattern of secretion synchronized with that of LH (Licinio et al., 1998). Direct evidence in rodents has shown that the release of gonadotropin releasing hormone (GnRH)
was stimulated by leptin from isolated hypothalamic explants (Yu et al., 1997) and pulsatile LH release was inhibited by administration of a leptin antibody (Carro et al., 1997).

Blood glucose concentrations are normally twice as high (11 mM) in birds, including chickens, in comparison to mammals, stable, and decrease only slightly (ca 10%) after short term or prolonged fast in chickens (Belo et al., 1976; Simon and Rosselin, 1978; Rabeja et al., 1986; Beccavin et al., 2001) and turkeys (Anthony et al., 1990; Kurima et al., 1994). Chickens are highly resistant to insulin injections (Cramb et al., 1982; Simon, 1989; Akiba et al., 1999). Insulin concentrations are increased in the fed state and decreased in the fasting state in chickens and turkeys (Simon and Rosselin, 1978; Anthony et al., 1990). Insulin has been shown to have an up-regulating effect on tissue specific leptin expression in broiler breeder hens (Ashwell et al., 1999). Chicken leptin expression was increased by insulin injection for 4 d in liver but not in adipose tissue, which agrees with the studies in mammals that circulating leptin concentrations and leptin mRNA expression were increased by insulin (Cohen et al., 1996; Ashwell et al., 1999).

Production of settable eggs is improved by feed restriction in comparison to ad
libitum-feeding of broiler breeder hens, but the relationships among ovulation, oviposition, interval between LH surges, leptin, and insulin concentrations have not been reported. The aim of the current study was to better document peripheral leptin and insulin concentration changes during ovulatory cycles when broiler breeder hens were fed under different feeding programs early and late in the reproductive period. Feeding programs were 1) ad libitum-fed or 2) restricted-fed during growth and the reproductive periods, or 3) restricted-fed during growth period and then switched to ad libitum-fed during the reproductive period.

MATERIALS AND METHODS

Birds and Blood Sample Collection

Broiler breeder hens (Ross 508) were raised in floor pens with continuous lighting (24L:0D) and with ad libitum access to feed until 1 wk of age. The hens were separated into 2 duration of reproduction groups and 3 feeding program groups (Table 4.1). Hens were sampled early (Early), at the peak of egg production, and late (Late), after egg production declined approximately 25%. The first group of hens was ad libitum fed during both growth and reproductive periods (FF). The second group of
hens was restricted-fed during growth and then switched to ad libitum feeding at 22 wk of age when photostimulated (RF). The third group of hens was restricted fed during both growth and reproductive periods (RR). The program of feed restriction used in the second and third groups was based on recommendations of the supplier (Aviagen, 2000).

All hens were given a 8L:16D photoperiod from 1 to 22 wk of age. All hens were housed in cages (32X42X46 cm) with slatted rubber floor mats and photostimulated with 16L:8D to induce sexual maturity at 22 wk of age. All hens were switched to a 24L:0D photoperiod to allow LH surges to free run immediately following cannulation and 3 to 5 d before serial blood sampling began. The cannulation procedure and success rate of complete sample collection (hourly for 240 h) for the hens is described in detail in Chapter 2 (Liu et al., 2004a). All hens were given feed at 0700 h during 10 d of serial bleeding. Some of the ad libitum-fed hens of the FF and RF groups ate feed immediately after feed was given as did all of the restricted-fed hens of the RR groups.

Blood samples were taken hourly (1.5 mL) for 240 h early (Early, 3 to 6 wk of production) in the reproductive period and after production had declined by about 25% (Late, 20 to 30 wk of production; Table 4.1).
**LH, Leptin, and Insulin Assays**

Concentrations of LH were measured hourly for 240 h by radioimmunoassay (RIA) as previously described using 100 µL plasma for each duplicate (Proudman et al., 1984; Bacon and Long, 1996). The LH intrassay and interassay coefficients of variation (CV) of plasma pools from laying broiler breeder hens with concentrations of LH (mean±SD=3.10±0.21 ng/mL) were 7.3 % and 6.9 %, respectively.

Concentrations of leptin were measured in samples collected every 4 h by RIA as described using 100 µL plasma for each duplicate (Evock-Clover et al., 2002; McMurtry et al., 2003). The leptin intrassay and interassay CV of plasma pools from laying broiler breeder hens were 3.7 % and 4.4 %, respectively.

Concentrations of insulin were measured in samples collected every 4 h by RIA as described using 200 ul plasma for each duplicate (McMurtry et al., 1983). Antibody for chicken insulin was prepared in female guinea pigs (McMurtry et al., 1983). The insulin intrassay and interassay CV of plasma pools from laying broiler breeder hens were 1.2 % and 3.9 %, respectively.
Statistical Analyses

The LH raw data were evaluated by the Pulsar algorithm to identify LH and P₄ preovulatory surges, and to calculate baseline, overall, and peak amplitude concentrations, and preovulatory surge durations of LH (Merriam and Wachter, 1982). For both LH, the G-values used for Pulsar analysis were G(1)=50, G(2)=2.6, G(3)=1.9, G(4)=1.5, G(5)=1.2. The standard deviations for LH used for PULSAR analyses was (7.27X)/100, where X is the sample concentration measured in an individual sample. The model for these analyses was a 2X3 factorial with interaction. Data were analyzed by ANOVA with main effects: duration of egg production (Early and Late), and feeding level (FF, RF, and RR), and their interaction. Individual hen was error term for all statistical analyses. Where appropriate, Tukey’s test was used to compare means. The following measurements were analyzed: 1) body weight after serial bleeding; 2) liver weight; 3) fat pad weight; 4) overall mean concentrations of leptin and insulin; 7) baseline concentrations of LH; 8) peak amplitude concentrations of LH; and 9) interval between preovulatory surges of LH. In addition, Pearson correlation coefficients were calculated among all measurements. The MINITAB¹ software program was sued for all statistical analyses.

¹ MINITAB, State College, PA 16801-3008
RESULTS

Body weight was heavier in the Late hens than in the Early hens, and among feeding programs (FF>RF>RR; Table 5.1). Liver weight was heavier in the Early-RF hens than Early-FF and Early-RR hens, and heavier in the Late-FF and Late-RF hens than in the Late-RR hens. Abdominal fat pad weight was not different between Early-FF and Late-FF hens but was heavier in the Late-RF and Late-RR hens than in Early-RF and Early-RR hens, respectively. The interval between LH surges was longer in the Late hens than in the Early hens but was not different among feeding programs. Leptin concentrations were not different between the Early and the Late hens or among feeding groups. Insulin concentration was higher in the Early-RR hens than in the Early-FF hens, but not different among feeding programs in the Late hens. Insulin concentrations also decreased in the Late-RR hens in comparison to the Early-RR hens. The highest and lowest CV of leptin concentration were found in Late-FF and Early-RF hens, respectively (Table 5.2).

Patterns of free running preovulatory LH and leptin and insulin concentrations of representative hens over the 10 d of serial bleeding from each group are shown in
Figure 5.2. Hens #12, 40, 45, 62, 88, and 127 had the shortest intervals between LH surges within their group (top of each panel). Hens #5, 26, 47, 66, 85, and 128 had intervals between LH surges closest to the mean within their group (middle of each panel). Hens #16, 32, 60, 72, 86, and 126 had the longest intervals between LH surges within their group (bottom of each panel). The LH surges, not associated with an oviposition, are called blind surges (i.e. Early-FF hen #5, Early-RF hen #26, and Late-FF hen #66). A possible explanation for blind surges is internal ovulation. Leptin and insulin concentrations fluctuated during ovulatory cycles independently from preovulatory LH surges (Fig 5.1). In general, insulin concentrations were much more stable in the FF and RF than in the RR hens, in whom insulin concentrations increased after daily feeding or daily meal feeding, respectively (Figure 5.1c and f). The Early-RR hen #47 had very low leptin concentrations during the last 2 d of 10 d of serial bleeding (Figure 5.1c). This hen had heaviest abdominal fat pad weight (126.4 g) in comparison to the other RR hens (mean±SE=88.3±15.8 g). The Late-FF hens #62 and #72 had very low leptin concentrations during ovulatory cycles (Figure 5.1 d). These 2 hens had heavier fat pad weight of 179.4 and 266.8 g, respectively.

Correlations of leptin and insulin concentrations and body weight, LH baseline
and LH amplitude concentrations, interval between LH surges, E2 concentrations, liver and abdominal fat pad weights, and duration of the reproductive period are listed in Table 5.3. Leptin and insulin were not correlated, nor were they correlated with any of the other measurements (Table 5.3).

**DISCUSSION**

Ad libitum feeding during both the growth and the reproductive periods resulted in heavier body weight, excessive body fat deposition, and a decrease in production of settable eggs in comparison to restricted-fed broiler breeder hens (Robinson et al., 1991; Yu et al., 1992; Chapter 4). Several studies have reported that female reproductive efficiency is modified by several factors such as age, duration of the reproductive period, duration of follicular development, nutritional state, and body weight or abdominal fat pad weight in laying hens. The interval between preovulatory LH surges is mainly associated with the difference in egg production early and late in the reproductive period in turkey hens (Liu et al., 2001, 2002) and broiler breeder hens (Chapter 4). The relationship among egg production rate, frequency of preovulatory LH surges, progesterone and estradiol-17ß concentration have been reported in detail in
Chapter 4. Leptin is thought to be important for successful reproduction in mammals (Barash et al., 1996; Cheung et al., 1997; Gruaz et al., 1998), but its importance is still controversial in avian species (Taouis et al., 1998; Friedman-Einat et al., 1999; Taouis et al., 2001; Richards, 2003).

The chicken leptin gene has been cloned and sequenced by Taouis et al. (1998) and was confirmed by Ashwell et al. (1999). They reported that the sequence of the coding region of chicken liver and adipose tissue cDNA is >95% identical to the leptin gene of rat and mouse. A later study (Friedman-Einat et al., 1999) failed to confirm the presence of the leptin mRNA reported by Taouis et al. (1998) in adipose tissue and liver of chicken, turkey, goose, or Japanese quail. A decrease in feed intake was found in young chickens (5 wk of age) after intravenous or intraperitoneal injection of chicken leptin (Dridi et al. 2000), but not found in chickens injected with mouse leptin (Bungo et al., 1999), suggesting mouse leptin may not bind to the chicken leptin receptor, or that the mechanism of feed intake regulation in chicken may be different from mammals. Leptin concentrations measured in the current study were not associated with body or abdominal fat pad weights, and thus circulating leptin may be not be active in the regulation of feed intake and energy balance in broiler breeder hens.
Studies have been reported where the effects of acute starvation-refeeding or acute leptin treatment on feed intake were measured. Leptin concentration increased after acute refeeding and after over feeding and decreased after acute fasting in mammals (Boden et al., 1996; Marie et al., 2001). Also, reduced feed intake was seen after injection of recombinant chicken leptin (Dridi et al., 2000; Lohmus et al., 2003) and mouse leptin (Denbow et al., 2000) in chickens. The protocols for the refeeding studies were different from the current study where chronic feed restriction rather than acute starvation was applied. Heavier body and heavier fat pad weights were induced by long term ad libitum feeding in comparison to long term restricted feeding. Fat pad weight was positively associated with body weight ($r=0.70, \ P=0.001$). Higher leptin concentrations, probably secreted by adipocytes, have been reported in mammals (Pelleymounter et al., 1995; Campfield et al., 1996) and chicken (Ashwell et al., 2001). Even though abdominal fat pad weight was changed by feeding program in broiler breeder hens and changed with duration of the reproductive period in the RF and RR groups of hens, no difference was found in leptin concentrations, nor was leptin related to changes in hormone related to reproduction, suggesting that leptin is not an important factor in controlling egg production rate in chickens.
Insulin participates in the long term signaling of satiety to hypothalamus to control metabolism and reproduction (Bruning et al., 2000). The release of insulin has been reported to be controlled by meal times rather than a circadian rhythm. The current study examined several hundred preovulatory LH surges in broiler breeder hens and unlike insulin concentrations, the occurrence of LH surges is highly dependent on the photoschedule, usually occurring during the later portion of the scotophase. Insulin concentrations increased immediately after feeding the RR hens and declined to basal levels after several h in comparison to the FF hens where insulin concentrations were relatively constant. Insulin has been reported to increase leptin mRNA and peripheral leptin concentrations in rodents and humans (Cohen et al., 1996). The current study did not agree that increasing leptin concentration was dependent on an increase in insulin concentration after feeding. Insulin and leptin concentrations were not correlated with each other or with any LH measurements, suggesting both leptin and insulin hormones are not important factors in the control of rates of ovulation/oviposition and egg production in broiler breeder hens.

Abdominal fat pad weight was only correlated with body weight ($r=0.70$, $P<0.001$) in laying hens but not with the hormones related to reproduction, suggesting
that adiposity is not a signal to stimulate the hypothalamus to release GnRH or the anterior pituitary to release LH, and thus alter egg production rate. Taken together, it was concluded that the abdominal fat pad weight, and leptin and insulin concentrations were not important contributors to the settable and abnormal egg productions among feeding programs early and late in the reproductive period in broiler breeder hens.

REFERENCES


Bacon, W.L., and D.W. Long, 1996.  Secretion of luteinizing hormone during a forced in

Clifton, and R.A. Steiner, 1996.  Leptin is a metabolic signal to the reproductive
system.  Endocrinology 137:3144-3147.

Recombinant porcine leptin reduces feed intake and stimulates growth hormone

Insulin-like growth factors and body weight in chickens divergently selected for


Bjorbaek, C., and B.B. Kahn, 2004.  Leptin signaling in the central nervous system and

Blache, D., R.L. Tellam, L.M. Chagas, M.A. Blackberry, P.E. Vercoe, and G.B. Martin,
2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal


Krone, D. Muller-Wieland, and C.R. Kahn, 2000.  Role of brain insulin receptor

Bungo, T., M. Shimojo, Y. Masuda, T. Tachibanab, S. Tanaka, K. Sugahara, and M.


Mantzoros CS, J.S. Flier, and A.D. Rogol, 1997. A longitudinal assessment of
hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. Clin. Endocrinol. Metab. 82:1066-1070.


Table 5.1. Main effect means±SE for body, liver, and abdominal fat pad weights, luteinizing hormone (LH) surge interval, and concentrations of leptin and insulin in broiler breeder hens early (Early) and late (Late) in the reproductive period and among feeding programs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Duration of reproduction$_1$</th>
<th>Feeding program$_2$</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>4.35±0.08$^b$</td>
<td>4.93±0.07$^a$</td>
<td>0.00</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>117.4±3.8</td>
<td>111.5±3.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Fat pad weight (g)</td>
<td>143.0±10.0$^b$</td>
<td>191.4±9.3$^a$</td>
<td>0.00</td>
</tr>
<tr>
<td>LH interval (h)</td>
<td>31.19±1.60$^b$</td>
<td>43.48±1.52$^a$</td>
<td>0.00</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>3.40±0.23</td>
<td>3.03±0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>2.93±0.10</td>
<td>2.92±0.09</td>
<td>0.97</td>
</tr>
</tbody>
</table>

$^a$$^b$$^c$Means with different superscript letters within rows and main effect are different (P<0.05).

$^1$Early hens were sampled at peak of egg production (3 to 6 wk egg production). Late hens were sampled after egg production had declined by about 25% (20 to 30 wk of egg production).

$^2$The FF hens were ad libitum-fed during growth and reproductive periods. The RF hens were restricted-fed during growth and then switched to ad libitum feeding at 22 wk of age when photostimulated. The RR hens were restricted-fed during both growth and reproductive periods. The feed restriction program used in the RF and RR hens was based on recommendations of the supplier.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Early-FF&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Early-RF&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Early-RR&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Late-FF&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Late-RF&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Late-RR&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>5.11±0.14</td>
<td>4.17±0.14</td>
<td>3.74±0.12</td>
<td>5.46±0.12</td>
<td>4.75±0.12</td>
<td>4.55±0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>107.3±6.9&lt;sup&gt;de&lt;/sup&gt;</td>
<td>140.1±6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.7±6.0&lt;sup&gt;de&lt;/sup&gt;</td>
<td>131.4±6.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>116.7±5.8&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>86.5±6.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>Fat pad weight (g)</td>
<td>213.8±18.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126.9±18.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>88.3±15.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>203.3±16.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>196.8±15.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>174.2±16.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>LH interval (h)</td>
<td>32.4±3.1</td>
<td>30.3±2.9</td>
<td>31.0±2.7</td>
<td>40.8±2.8</td>
<td>47.3±2.56</td>
<td>42.4±2.7</td>
<td>0.29</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>3.82±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.29±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.09±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.57±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.05±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.46±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>2.30±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.12±0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.37±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.81±0.16&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.77±0.15&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.19±0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>Leptin CV (%)</td>
<td>31.3</td>
<td>27.6</td>
<td>44.4</td>
<td>52.9</td>
<td>36.8</td>
<td>42.7</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>a-e</sup>Means with different superscript letters within rows and main effect are different (<i>P</i>&lt;0.05).

<sup>1</sup>The Early FF hens were ad libitum-fed during growth and reproductive periods and sampled at peak egg production. The RF hens were restricted-fed during growth and then switched to ad libitum feeding at 22 wk of age when photostimulated. The RR hens were restricted-fed during both growth and reproductive periods. The feed restriction program used in the RF and RR hens was based on recommendations of the supplier.

Table 5.2 Interaction means±SE for body, liver and fat pad weights, luteinizing hormone (LH) surge interval, and concentrations of leptin and insulin, and coefficients of variation (CV) of leptin concentrations in broiler breeder hens early (Early) and late (Late) in the reproductive period and among feeding programs.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Body weight</th>
<th>LH baseline</th>
<th>LH amp.</th>
<th>LH interval</th>
<th>E\textsubscript{2} weight</th>
<th>Liver weight</th>
<th>Fat pad weight</th>
<th>Leptin</th>
<th>Duration of reprod.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>r=-0.02</td>
<td>P=0.88</td>
<td>r=0.13</td>
<td>r=0.15</td>
<td>r=-0.05</td>
<td>r=-0.07</td>
<td>r=0.00</td>
<td>r=0.09</td>
<td>--</td>
</tr>
<tr>
<td>Insulin</td>
<td>r=-0.13</td>
<td>P=0.30</td>
<td>r=0.16</td>
<td>r=-0.10</td>
<td>r=-0.03</td>
<td>r=-0.08</td>
<td>r=0.08</td>
<td>r=-0.14</td>
<td>r=-0.13</td>
</tr>
</tbody>
</table>

Table 5.3 Correlation (r) and statistical probability (P) among body weight, luteinizing hormone (LH) baseline concentration, LH amplitude (amp.) concentration, LH surge interval, estradiol-17\beta (E\textsubscript{2}) concentration, liver and fat pad weights, leptin and insulin concentrations, and duration of the reproductive period (reprod.).
Figure 5.1. Hourly plasma luteinizing hormone (LH), and leptin and insulin concentrations every 4 h for 240 h in representative broiler breeder hens. Data presented are for hens with the shortest, closest to the mean, and longest surge intervals for each experimental group, which are shown at the top, middle, and bottom of each panel (a-e), representatively. Closed circles at the top of the panels represent the approximate times of oviposition of settable eggs based on palpation and egg collection records. Open circles at top right (i.e., Early-FF hen #5) or left (i.e., Early-FF hen #16 and Late-FF hen #72) of some panels represent oviposition of soft shelled eggs or of a soft shelled egg (SS; i.e. Early-FF hen #5 and hen #15) or of a hard shelled egg (HS; i.e., Early-FF hen #12 and Early-RF hen #32) in the uterus at necropsy. Arrows give assigned association of an LH surge to the oviposition of an egg based on palpation and egg collection records. Surges not associated with ovipositions are called blind LH surges. The question mark(s) in some panels indicate blind LH surges (i.e. Late-FF hen #66).
Fig 5.1: Continued

A

Early-FF #12

Early-FF #5

Early-FF #16

A (Continued)
Fig 5.1: Continued

B

Early-RF #40

Early-RF #26

Early-RF #32

B (Continued)
Fig 5.2: Continued

C

Early-RR #45

Early-RR #47

Early-RR #60

C (Continued)
Fig 5.1: Continued

Late-FF#62

Late-FF #66

Late-FF #72

D (Continued)
Fig 5.1: Continued

Late-RF #88

Late-RF #95

Late-RF #96

E (Continued)
Fig 5.1: Continued

Late-RR #127

Late-RR #128

Late-RR #126

F (Continued)
CHAPTER 6

DURATION OF FOLLICULAR DEVELOPMENT INCREASES WITH ADVANCING DURATION OF THE REPRODUCTIVE PERIOD IN BROILER BREEDER HENS

ABSTRACT

Production of settable eggs is increased by feed restriction, but declines as the duration of the reproductive period progresses in broiler breeder hens. It was hypothesized that the decrease in egg production with advancing duration of the reproductive period and ad libitum feeding is associated with a longer duration of follicular development. The objective of the current study was to document the duration of ovarian follicular development early (Early, at 3 to 6 wk of egg production) and late (Late, after egg production had declined by about 25% at 19 to 31 wk of egg production) in a reproductive period in broiler hens. A group of hens was ad libitum-fed during both growth and reproductive periods (FF), a second group of hens was restricted-fed during
growth but ad libitum-fed after photostimulation (RF), and a third group of hens was restricted-fed during both growth and reproduction periods (RR) to induce different egg production rates and degrees of adiposity early and late in the reproductive period. The hens were photostimulated with 16L:8D at 23 wk of age and were then switched to 24L:0D after cannulation (2 wk after starting dye feeding) to allow luteinizing hormone surges to free run (see Chapter 3). Sudan IV (R; 100 mg hen\(^{-1}\)d\(^{-1}\)) and Sudan Black B (B; 50 mg hen\(^{-1}\)d\(^{-1}\)) were suspended in canola oil and 1.0 mL hen\(^{-1}\)d\(^{-1}\) of one of the suspensions was force-fed by gavage 1 h before feeding the hens to monitor the duration of follicular development. The total (settable plus abnormal) egg production rate (74 to 84\% and 52 to 56\%, respectively for Early and Late periods) was not different between feeding treatments within the Early and Late hens. The duration of ovarian follicular development (8.7 to 9.1 d and 9.8 to 10.2 d, respectively) was not different between feeding treatments within Early and Late hens. Early hens in each feeding treatment had a higher total egg production rate but shorter duration of follicular development in comparison to Late hens. It was concluded that the longer duration of ovarian follicular development in Late hens was associated with a lower total egg production rate but that feed restriction had no effect on duration of ovarian follicular development in broiler
INTRODUCTION

Production of settable eggs by broiler breeder hens is increased by feed restriction through growth and reproductive periods in comparison to ad libitum-feeding (Leeson and Summers, 1983; Robinson et al., 1991; Yu et al., 1992a). Ad libitum-fed broiler breeder hens had more multiple (difference in weight of similar follicles less than 1 g) hierarchical follicles and laid more abnormal eggs (double yolked, soft shelled, thin shelled, and extracalcified) compared to restricted-fed hens (Jaap and Muir, 1968; Renema et al., 1999; Yu et al., 1992b). Total (settable and abnormal) egg production of restricted-fed hens peaked at about 85% and then gradually declined by about 1% weekly to 60% at 30 wk of production whereas ad libitum-fed hens peaked at about 70% and then declined 1% weekly to 50% at approximately 20 wk production (Figure 4.1). The pattern of total egg production of ad libitum-fed hens switched from feed restriction to ad libitum feeding when photostimulated at 22 wk of age was between restricted-fed and ad libitum-fed hens (Figure 4.1).

Turkey hens start to lay eggs about 3 wk after photostimulation (Bacon et al., 1972; Bacon et al., 2002), suggesting it takes about 3 wk for a nonfunctional ovary to
develop to a functional state in photosensitive turkey hens after photostimulation. After photostimuation (about at 22 wk of age), the time for broiler breeder hens to reach sexual maturity is variable, depending on feeding treatment (Eitan et al., 1998; Bruggeman et al., 1999). It takes about 3 wk to reach sexual maturity for ad-libitum fed broiler breeder hens and 4 to 5 wk for restricted-fed hens, suggesting feeding treatment leading to differences in body weight and adiposity modified the time to attain sexual maturity after photostimulation. During this time, ad libitum fed hens gained little body weight, but restricted fed hens switched to ad libitum feeding gained considerable weight, and restricted-fed hens gained weight as their feed allowance was increased at photostimulation.

Early in the reproductive period, a functional ovary of broiler breeder hens contains approximately 50 small (<1 g) follicles and approximately 10 hierarchical yolky follicles (>1 g), but there is a decrease in the number of hierarchical follicles as the duration of the reproductive period progresses (Bahr and Palmer, 1989). There is a strong negative association between body weight and egg production rate between different strains of turkeys (Bacon et al., 1972; Liu et al., 2001b), and in broiler breeder hens of the same strain but fed to achieve different body weights (Robinson et al., 1991;
Turkey hens of a heavy body weight line (RBC3) had a greater number of hierarchical yolky follicles (11.3) but poorer rate of egg production whereas the hens of light body weight line (Egg) had a lower number of hierarchical follicles (7.6) but excellent egg production early in the reproductive period (Liu et al., 2001b). Very similar results were observed in chicken hens; light-weight commercial egg producing strains of chickens with excellent egg production have fewer hierarchical follicles than heavier weight broiler breeder strains (Williams and Sharp, 1978; Yu et al., 1992b; Renema et al., 1999; Hocking and Robertson, 2000). During follicular development, small follicles are highly susceptible to undergo apoptosis and atresia whereas hierarchical yolky follicles are resistant to undergo atresia (Gilbert et al., 1983; Waddington et al., 1985; Johnson, 1996). Egg production rate may be regulated by the duration of follicular development and modified by the incidence of atresia of hierarchical follicles (Gilbert et al., 1983; Waddington et al., 1985; Yu et al., 1992b).

Injecting (Zakaria et al., 1984a, b; Palmer and Bahr, 1992) or feeding (Gage and Gage, 1908; Bacon and Cherms, 1968; Yu et al., 1992b) different fat-soluble dyes (Sudan black and Sudan IV) has been used to examine the duration of rapid follicular development in laying hens. This method is relatively crude, however, and duration of
rapid follicular development can only be determined to the nearest day. A decrease in the number of hierarchical follicles and an increase in follicle size (yolk volume) are coincident with advancing duration of the egg production period (Williams and Sharp, 1978; Zakaria et al., 1983; Yu et al., 1992b). Four factors, including a higher rate of atresia of hierarchical follicles (Palmer and Bahr, 1992), longer duration of follicular development of hierarchical follicles (Bacon et al., 1972), faster uptake of yolk into hierarchical follicles with the same duration of follicular development a longer interval between follicles entering the hierarchy, or a combination of these factors, might result in fewer but larger hierarchical follicles in hens late in comparison to early in the reproductive period. The duration of rapid follicular development has been estimated to be from 6 to 18 d for chickens (Zakaria et al., 1983; Yu et al., 1992b), quail (Bacon et al., 1973, Grau, 1976), pigeons (Cuthbert, 1945; Birrenkott et al., 1988), turkeys (Bacon and Cherms, 1968, Hocking et al., 1987), and geese (Grau, 1976). A longitudinal study has shown that in laying Leghorn hens the length of the rapid development period of hierarchical follicles was 8.5 d at 7 months of age and decreased to 8.2 d at 19 months of age (Zakaria et al., 1983), but the weight of the F1 hierarchical follicle increased from 12.5 to 15.5 g, suggesting that an increased efficiency of yolk recruitment into rapidly
growing hierarchical follicles may contribute to the larger follicle size at the older age.

Yu et al. (1992b) reported that the duration of rapid follicular development from hens at 34 wk of age was longer (9.6 d) in ad libitum-fed than in restricted-fed and ad libitum-fed switched from restricted-fed broiler breeder hens (8.4 d).

The rest period is defined as the interval between maturation of a follicle, indicated by last d of dye deposition, and d of oviposition of an egg (Bacon and Cherms, 1968). The rest period may be lengthened by holding of an egg in the uterus longer than necessary for shell and cuticle deposition. There is a negative relationship between the rest period and egg production rate. First eggs of clutches also had a longer rest period than subsequent eggs in clutches. The rest period was estimated to range from 1.5 to 1.7 d for pigeon and guinea fowl hens (Birrenkott, 1988) and from 1.4 to 1.9 d for turkey hens (Bacon and Cherms, 1968).

The 6 trials of the current study were designed to examine the duration of rapid follicular development when egg production rate was expected to be different due to duration of the reproductive period and feeding program. The hens were also serially bled hourly to monitor peripheral concentrations of luteinizing hormone, progesterone, estradiol-17β, leptin, and insulin while the hens were under daily dye feeding treatment.
These hormonal data are presented separately in Chapters 4 and 5. It was hypothesized that the duration of rapid follicular development of hens would be longer with advancing duration of egg production, but would not be affected by feeding program either early or late in the reproductive period.

**MATERIAL AND METHODS**

**Broiler Breeder Hens and Management**

The current study consists of 6 trials (Liu et al., 2004a). Female broiler breeder hens (Ross 508) were given a 24L:0D photoperiod and ad libitum-fed until 1 wk of age. The hens were then separated into 3 feeding program groups and were studied either early (Early, 3 to 6 wk of egg production) or late (Late, egg production had declined 25% compared to each treatment group of Early hens) in the reproductive period (Table 6.1): The feeding program groups were: 1) ad libitum-fed during growth and reproductive periods (FF), 2) restricted-fed during growth and then switched to ad libitum feeding when the hens were photostimulated at 22 wk of age (RF), and 3) restricted-fed during growth and reproductive periods (RR). The hens were housed in floor pens until 1 wk before photostimulation and then housed in individual cages (32X42X46 cm) at 21
wk of age. Feed restriction was based on the recommendation of the primary breeder for this line of broiler breeder hens (Avigen, 2000). The hens were given a photoschedule 6L:18D between 1 and 22 wk of age before they were switched to a photoschedule of 16L:8D, to induce sexual maturity. All hens were switched to 24L:0D photoperiod following cannulation to allow preovulatory surges of luteinizing hormone (LH) and circadian rhythms to free run for Early and Late hens (Table 6.1): FF, 4 and 19 wk of egg production, respectively; RF, 3 and 22 wk of production, respectively; RR, 5 and 30 wk of production, respectively. Peripheral hourly patterns of LH, progesterone, estradiol-17β, leptin, and insulin concentrations were reported separately in Chapters 4 and 5. The hens used in each trial needed to meet the requirement that before cannulation all hens laid eggs for the previous 2 wk. Egg production of individual hens was recorded daily. Early RF and RR hens started laying 3 and 4 wk after photostimulation but some of the Early FF hens started laying 2 wk before photostimulation (at 20 wk of age). All the hens were necropsied immediately after 10 d of hourly blood sampling. Hierarchical follicles were removed by blunt dissection, weighed, and then placed in boiling water for 15 min, cooled in tap water for 15 min, and then cut in half to determine the dye ring number and sequence.
Dye feeding treatment

Sudan IV (R) and Sudan Black B (B) were suspended in canola oil (100 and 50 mg mL\(^{-1}\), respectively). The sequence of daily dye feedings was R,B,R,B,R,B,R,B,R,B,R,B,R,B,B,R,B,R,R,B,R,B,R,B,R,B,R,B,R,B,R,B,B,R,B,R,B,R,B,B,R,B,R,B,R,B,B,R,B,R,R,B,R,B,R,B,R,B,R,B,R,B,B,R,B,R,B,R,B,B,R,B,R for the Early FF hens, and B,R,B,R,B,R,B,R,B,R,B,R,B,B,R,B,R,R,B,R,B,R,B,R,B,R,B,B,R,B,R,R,B,B,R,B,R,B,R,B,B,R,B,R,B,B,R,B,R,B,R,B,R,B,B,R,B,R for the other groups, respectively. The time for the dye rings to reach the center of the yolk was estimated to be about 2 wk. Starting 2 wk before cannulation, the hens were daily restrained and gavaged with either Sudan IV (R) or Sudan Black B (B) at 0800 h, about 1 h before feeding the restricted-fed hens, using a 1 ml syringe with a rubber tube (14 cm long, 0.4 cm i.d. x 0.7 cm o.d.) attached. The rubber tube was passed down the esophagus to the level of the crop. Eggs of individual hens were recorded and collected daily. Abnormal eggs were recorded as well. The eggs were weighed and then hard boiled for approximate 30 min, then cooled in tap water for 15 min, the yolk removed, weighed, cut in half, and the sequence of dye rings recorded. The sequence of dye rings in each ovum and follicle was observed and determined by same person throughout the experiment. The dye ring sequence was recorded without the knowledge of the dye ring
sequence from previously laid eggs or follicles.

Statistics

Data were analyzed by ANOVA with a factorial arrangement of treatment with main effects, duration of the reproductive period (Early and Late) and feeding program (FF, RF, and RR) and their interaction, to determine statistical differences (P<0.05) in duration of follicular development (measured by number of dye rings) and rest period (period of time between d of last dye ring deposition and oviposition). Correlations were calculated among all measurements. Individual hen was error term for all statistical analyses. The MINITAB\(^1\) computer program was used for the statistical analyses.

RESULTS

The last dye ring deposited in yolk was on the periphery and the earliest in the center. Figure 6.1a shows that the earliest dye ring was not deposited in the center of this yolk (the sequence from center to periphery: no dye-BRBRBRBBR) whereas Figure

\(^1\) MIMITAB, State College PA, 16801-3008
6.1b shows deposition of dye to the center of this yolk and the duration of follicular development was 11 d (the sequence from the center to periphery: BRBBRBRBBR). Dye ring sequences of eggs oviposited during the 10 d period of serial blood sampling of representative Early and Late hens of all the feeding groups are shown in Figure 6.2 a, b, c, d, e, and f. The representative hens from each feeding group were selected based on the mean interval between LH surges for laying hen data presented in the top panels (see Chapter 4) or number of hierarchical follicles at necropsy in the bottom panels. The hens with shortest interval between LH surges or the greatest number of hierarchical follicles at necropsy are presented on the left side of each panel. The hens with the interval between LH surges closest to the mean for each feeding group or closest to the mean number of hierarchical follicles at necropsy are presented in the middle of each panel. The hens with the longest interval between LH surges or the lowest number of hierarchical follicles at necropsy are presented on the right side of each panel (Figure 6.2).

The interval between LH surges and the duration of follicular development in the representative laying hens are given in Table 6.2. A longer interval between LH surges was not always associated with a longer duration of follicular development, i.e.,
see Early-RF hen # 32, Figure 6.2b. Most follicles selected into the hierarchy initiated rapid development 1 d later than the previously selected follicle, but the rest period was not necessarily constant in a clutch (hen #5 in Figure 6.2a). Examples of two follicles being selected into the hierarchy on the same d but with different durations of follicular development were noted. This resulted in different times of ovulation and oviposition of eggs within a clutch (hens #9 and #12 in Figure 6.2a, hen #40 in Figure 6.2b, hen #45 in Figure 6.2c, hen #88 in Figure 6.2e, hen #128 in Figure 6.2f) or between clutches (hen #26 in Figure 6.2b, hen #83 in Figure 6.2d). Some cases were noted where 2 follicles were selected into the hierarchy on the same day and had the same duration of follicular development but the subsequently oviposited egg had a longer rest period, i.e., hen #26 in Figure 6.2b, hen #45 in Figure 6.2c, and hen #62 in Figure 6.2d. The Late RF hen #86 had a very long LH surge interval (mean 85.5 h) but the ovum did not have an increased duration of follicular development (Figure 6.2 e). The egg oviposited on Day 8 was selected into the hierarchy only 2 d after the previous ovum had been selected into the hierarchy. These two ova had the same duration of follicular development (12 d) but the second ova had a very long rest period (4 d) due to the hen holding the hard shell eggs for several d, according to palpation records (Fig 6.2e).
The LH surge interval, duration of follicular development, and number of hierarchical follicles in representative hens (see Figure 6.2a-f) are listed in Table 6.2. The shortest LH surge interval was not always associated with shortest duration of follicular development (Late-RR hen #127) and greatest number of hierarchical follicles (Late-FF #62). The LH surge interval and number of hierarchical follicles, identical dye sequence, multiple hierarchies, and follicular development stage at necropsy in representative hens (Figure 6.2a-f) are listed in Table 6.3. The hens with shortest LH surge interval had greater number of identical dye ring sequences and multiple hierarchies whereas the hens with longest LH surge interval had least number of identical dye ring sequences and multiple hierarchies.

Multiple (double or triple) hierarchical follicles were defined as the difference of weight between follicles of less than 1 g. Nine of 10 Early-FF hens and 7 of 12 Late-FF hens had multiple hierarchical follicles. Seven of 10 Early-RF hens and 6 of 14 Late-RF hens had multiple hierarchical follicles. Only 2 of 13 Early-RR hens but none of the Late-RR hens had multiple hierarchical follicles. Ad libitum-fed hens had more multiple hierarchical follicles in comparison to restricted-fed hens (Table 6.4, Figure 6.2a). Generally, the multiple hierarchical follicles had the same duration of follicular
development, but a few cases showed that these follicles were selected into hierarchies on different days but had weights < 1 g different at necropsy (Figure 6.2a, and e). The relationship between weight of hierarchical follicles and dye ring pattern of follicles are shown by representative hens from each feeding group (Fig 6.2a-f). The representative hens with the largest number, the mean number, and smallest number of hierarchical follicles are shown on the left, center, and right side of each panel, respectively. The pattern of dye ring sequence for each ovarian hierarchical follicle is plotted from the periphery of the ovum to the center. The same number and sequence of dye rings among follicles indicates that two or more follicles had same follicular development stage, i.e. Early-FF hens #4, #5, #16, Early-RF hens #39, #32, #26, Early-RR hens #45, #47, Late-FF hens #67, #61, and Late-RF hens #88, #93. The same follicular development stage was not always associated with similar weights of hierarchical follicles (Figure 6.2). A few cases show that 2 follicles had the same duration of follicular development but that the smaller follicle was selected into hierarchy one d earlier than the heavier follicle [Early-FF hen #5 (Figure 6.2a), Late-RF hens #88 and #93 (Figure 6.2e)]. The hens with a greater number of hierarchical follicles had a higher incidence of multiple hierarchical follicles in comparison to hens with a lower number of hierarchical follicles.
in each feeding treatment group (Figure 6.2 a-f).

The Early-FF hen #4 had two triple hierarchical follicle sets and 1 quadruple follicle set. The FF hens had (Early-FF hen #4) more hierarchical and multiple hierarchical follicles in comparison to RR hens (Early-RR hen #60). The follicles of some hens had an irregular pattern of follicular development (Early-FF hen #5, Late-RF hen #88, and Late-RF hen #93). The second largest follicle (F₂) of these hens was selected into hierarchy prior to the F₁ follicle but these 2 follicles maintained the same duration of follicular development before they were ovulated, suggesting that these F₂ follicle had a slower rate of yolk deposition than F₁ follicles resulting in lighter follicle weight, but they had the same duration of follicular development.

Body weight was heavier in the Late hens than in the Early hens, and was affected by feeding program (FF>RF>RR; Table 6.4). No interaction between duration of the reproductive period and feeding program was detected for body weight (Table 6.5). Ovary weight without hierarchical follicles was not different between Early and Late hens, but the FF hens had a heavier ovary than the RF and RR hens. No interaction was found for ovary weight. An interaction was found for oviduct weight; the oviduct of the Early-FF hens weighed less than for all other groups of hens. The incidence of atretic
follicles did not change with duration of the reproductive period, but was higher in the FF hens than in the RF and RR hens. None of hens had atretic follicles in Early-RF, Early-RR, and Late-RR groups whereas one Late-RF hen had one atretic follicle. Five of 10 Early-FF hens had at least one atretic follicle. The number of hierarchical follicles did not change in FF and RR hens as the duration of the reproductive period increased, but decreased in the RF hens as the duration of the reproductive period increased. Weight of the F₁ follicles increased in association with duration of the reproductive period and age of the hens, and this increase was greatest in the Late-FF hens in comparison to the Early-FF hens. The number of follicles with identical dye ring sequences decreased with increasing duration of the reproductive period and was lowest in the RR hens. The number of multiple hierarchical follicles decreased in the Late hens and was lower in the RR hens than in the FF and RF hens. The number of follicle stages was highest in the Late-FF hens and lowest in the Early-FF hens (Table 6.5). Durations of follicular development and rest periods were longer in the Late than Early hens and not affected by feeding program.

Body weight was positively associated with ovary weight, number of hierarchical follicles and multiple hierarchies, duration of follicular development, rest
period, and duration of the reproductive period (Table 6.6). Ovary weight was not correlated with any other measurement. Oviduct weight was negatively associated with number of atretic follicles and positively associated with F₁ follicle weight and number of follicular development stages. Number of atretic follicles was positively associated (P=0.05) with number of multiple hierarchies and negatively associated with oviduct weight and number of follicular development stages. Number of hierarchical follicles was positively associated with body weight, number of identical dye sequences, multiple hierarchies, and follicular development stages but negatively associated with duration of the reproductive period. The F₁ follicle weight was positively associated with oviduct weight, number of identical dye ring sequences, follicular development stages, durations of follicular development, and the reproductive period but negatively associated with number of multiple hierarchy follicles. Number of identical sequence was positive associated with number of multiple hierarchy follicles and negatively associated with duration of the reproductive period. Number of multiple hierarchical follicles was positively associated with duration of follicular development but negatively associated with duration of the reproductive period. Duration of follicular development was positively associated with rest period and duration of the reproductive period.
DISCUSSION

Several ovarian follicular development and oviductal factors might affect the rate of settable egg production, including duration of rapid follicular development, duration of the rest period, rate of atresia of hierarchical follicles (Zakaria et al., 1983; Waddington et al., 1985; Bahr and Palmer, 1989), rate of selection into the hierarchy, incidence of blind surges of LH, and inappropriate oviposition time, or a combination of the above factors. Production of settable eggs was higher in Early-RR in comparison to Early-FF broiler breeder hens, but production of total (settable plus abnormal) eggs was not different among feeding treatments within Early and Late groups of hens, suggesting production of settable eggs was not controlled by the anterior pituitary (i.e. interval between LH surges; see Chapter 4). Duration of follicular development was not different among feeding treatments in Early and Late hens, but was longer in Late hens. This result is similar to interval between LH surges (see Chapter 4), suggesting the longer duration of rapid development may be positively associated with LH surge interval and negatively associated with production of total eggs. The current data show that Late hens had a longer duration of follicular development in comparison to Early hens,
suggesting the greater yolk volume deposited in the hierarchical follicle (heavier F₁ weight) was either at the same or a faster rate during rapid development. The factors resulting in higher production of abnormal eggs might be due to a dysfunctional oviduct associated with ad libitum-feeding. The dysfunctional oviduct might cause oviposition of soft shelled eggs instead of completely formed eggs.

There is a negative association between the number of hierarchical follicles and egg production rate in turkey (Bacon et al., 1972; Liu et al., 2001b) and broiler breeder hens (Yu et al., 1992b). Ad libitum-fed hens had more multiple hierarchical follicles in comparison to restricted-fed broiler breeder hens, similarly to large body size turkey hens (sire line) in comparison to small body size turkey hens (dam line; Melnychuk et al., 1997). A greater number of (multiple) hierarchical follicles would result in a better egg production rate in Early-FF hens than Early-RR hens if all hierarchical follicles selected into the hierarchy reach maturation, are sequentially ovulated and sequestered by the oviduct. The data, however, did not show a difference in total egg production between Early-FF and Early-RR hens. The higher rate of atresia in Early-FF hens may lower the potentially increased egg production rate from the greater number of follicles since the same duration of follicular development was found among Early and Late hens. The F₁
follicle weight was heavier in Early-RR hens than in Early-FF hens but the duration of follicular development was not different, suggesting a higher rate of yolk deposition in the follicles of RR hens, resulting in the larger F1 follicles in the Early-RR hens, and the same rate of production of total eggs in the hens. Since the Early-FF hens were 7 wk younger than the Early-RR hens, this difference may be related to hen age rather than feeding program.

Late-FF hens had a greater number of hierarchical follicles in comparison to Late-RR hens but the same F1 follicle weight, number of atretic follicles, and duration of follicular development, suggesting follicles were selected into the hierarchy and yolk deposited into the follicles at the same rate and egg production rate was slightly modified by atresia in Late hens. The pattern of preovulatory LH surges for abnormal (double yolked and soft shelled) eggs was not different from that of settable eggs (Chapter 4). Thus, 2 or more follicles might be ovulated at nearly the same time but with only one ovum entering the infundibulum which may explain the greater number of hierarchical follicles with same follicular development stage and atresia, and F1 weight in Late-FF hens with the same egg production rate in comparison to Late-RR hens.

Early-FF and Late-FF hens had a grater number of hierarchical follicles and a
greater number of multiple hierarchical follicles in comparison to RR hens, suggesting 2 or more follicles had the same duration of follicular development but multiple hierarchical follicles were not all selected to produce double or multiple yolked eggs. The number of follicular development stages is negatively associated with the number of multiple hierarchies. The current data show that the number of follicular development stages might not contribute to the poor egg production in Late hens in comparison to Early hens.

Poorer egg production may be partially attributed to longer rest periods between follicular maturation and oviposition. The decline in egg production with advancement of the reproductive period in each feeding treatment, however, was not due to longer rest periods. Hen #86 had a very long LH surge interval (85.5 h) but the duration of follicular development (11.8 d) was not consistent with the longest surge interval resulting in poor egg production rate (Table 6.2). This hen had a very long rest period (4.0 d) associated with this surge interval and held a hard shelled egg for several d. This hen had only 3 LH surges during the 10 d sampling period (Chapter 4). Taken together, the rate of follicular development in this hen was relatively constant even though a very long pause occurred due to the F1 follicle not continuing development but waiting for an
LH surge or some other unidentified signal to induce spontaneous ovulation. It is suggested that a longer duration of follicular development and a longer rest period (at least in some hens) might be important factors causing a decrease in egg production in the Late hens in comparison to the Early hens.

Follicles would have the same weight and have the same number of dye rings if the rate of yolk deposition into hierarchical follicles is constant and the follicles have the same duration of follicular development. Most ovarian data show that follicle weight is positively associated with the duration of follicular development in Early-RR and Late-RR hens. Some cases show that in FF and RF hens follicles with different weights had the same duration of follicular development (i.e. Early-FF hen #4) or similar weight (the difference <1.0 g) of the follicles had different duration of follicular development (i.e. Early-RF hen #32), suggesting the rate of yolk deposition into the follicle was not completely dependent on duration of follicular development.

Several hundred eggs were examined in the current study. Only 2 double yolked eggs were found, one in an Early-RF and one in a Late-FF hen, suggesting that a greater number of hierarchical follicles were not highly associated with production of double yolked eggs. The 2 ova in double yolked eggs had similar (the difference <1.0 g)
weights and the same duration of follicular development, suggesting these 2 follicles were selected into the hierarchy on the same day and had the same rate and duration of yolk deposition. The follicular development of the double yolked eggs was similar to the turkey (Bacon and Cherms, 1968). The mechanism, however, to control the development of these 2 follicles remains unclear but is probably controlled at the ovarian level.

In summary, production of total eggs was not different among Early and Late hens fed at different nutritional levels, but Early-RR hens laid more settable eggs in comparison to Early-FF hens. Early-RR hens had heavier F₁ follicle weights than Early-FF hens and Late hens had heavier F₁ follicle weights in comparison to Early hens. Early hens had a greater number of hierarchical follicles than Late hens. Duration of follicular development was not different among Early and Late hens fed at different nutritional levels, but was longer in Late hens. Early-FF hens had more atretic follicles than Early-RR hens but this was not found in Late hens. Early-FF and Late-FF hens had more multiple hierarchical follicles in comparison to Late-RR hens. The rest period was mildly modified the egg production egg in broiler breeder hens. It was concluded that longer duration of ovarian follicular development in Late hens was associated with lower
total egg production rate in broiler breeder hens, and that nutritional level had no effect on duration of follicular development.

REFERENCES


Gage, S.H., and S.P. Gage, 1908. Sudan III. Deposited in the egg and transmitted to the


<table>
<thead>
<tr>
<th>Variable</th>
<th>Early FF</th>
<th>Early RF</th>
<th>Early RR</th>
<th>Late FF</th>
<th>Late RF</th>
<th>Late RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hen (n)</td>
<td>10</td>
<td>10</td>
<td>13</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Age (wk)</td>
<td>25-26</td>
<td>29-30</td>
<td>32-33</td>
<td>43-44</td>
<td>49-50</td>
<td>58-59</td>
</tr>
<tr>
<td>Duration of production&lt;sup&gt;1&lt;/sup&gt; (wk)</td>
<td>4-5</td>
<td>3-4</td>
<td>5-6</td>
<td>19-20</td>
<td>22-23</td>
<td>30-31</td>
</tr>
<tr>
<td>Feeding&lt;sup&gt;2&lt;/sup&gt; (1-22 wk)</td>
<td>F</td>
<td>R</td>
<td>R</td>
<td>F</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Feeding&lt;sup&gt;2&lt;/sup&gt; (23 wk- end trial)</td>
<td>F</td>
<td>F</td>
<td>R</td>
<td>F</td>
<td>F</td>
<td>R</td>
</tr>
</tbody>
</table>

<sup>1</sup> The hens were photostimulated with 16L:8D at 22 wk of age and switched to 24L:0D to allow luteinizing hormone (LH) surges to free run when cannulated.

<sup>2</sup> All hens were ad libitum-fed until 1 wk of age. The hens were restricted-fed (R) based on the recommendation of the supplier (ROSS 508 broiler breeder hens) or ad libitum-fed (F) during growth (1 to 22 wk) and reproductive (22 wk to end of trial) periods.

<sup>3</sup> Hen was used early (E, at peak of production) or late (L, after production had declined by 25%) in the reproductive period.

Table 6.1.  Hen number, age, duration of production, and feeding treatments early (Early) and late (Late) in the reproductive period for the 6 trials.
<table>
<thead>
<tr>
<th></th>
<th>Early FF</th>
<th></th>
<th></th>
<th>Late FF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Representative hens</td>
<td>#12</td>
<td>#5</td>
<td>#9</td>
<td>#62</td>
<td>#66</td>
<td>#83</td>
</tr>
<tr>
<td>LH surge interval (h)</td>
<td>28.3</td>
<td>32.3</td>
<td>37.8</td>
<td>34.6</td>
<td>42.2</td>
<td>56.0</td>
</tr>
<tr>
<td>Follicular development (d)</td>
<td>8.3</td>
<td>9.8</td>
<td>8.6</td>
<td>9.0</td>
<td>10.0</td>
<td>10.3</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Early RF</td>
<td></td>
<td></td>
<td>Late RF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Representative hens</td>
<td>#40</td>
<td>#26</td>
<td>#32</td>
<td>#88</td>
<td>#85</td>
<td>#86</td>
</tr>
<tr>
<td>LH surge interval (h)</td>
<td>25.6</td>
<td>30.4</td>
<td>38.0</td>
<td>28.8</td>
<td>48.0</td>
<td>85.5</td>
</tr>
<tr>
<td>Follicular development (d)</td>
<td>8.6</td>
<td>10.0</td>
<td>9.0</td>
<td>10.4</td>
<td>10.4</td>
<td>11.8</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Early RR</td>
<td></td>
<td></td>
<td>Late RR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Representative hens</td>
<td>#45</td>
<td>#47</td>
<td>#60</td>
<td>#127</td>
<td>#128</td>
<td>#126</td>
</tr>
<tr>
<td>LH surge interval (h)</td>
<td>26.2</td>
<td>31.0</td>
<td>41.0</td>
<td>32.0</td>
<td>42.8</td>
<td>54.0</td>
</tr>
<tr>
<td>Follicular development (d)</td>
<td>9.0</td>
<td>8.6</td>
<td>9.0</td>
<td>10.3</td>
<td>9.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Hens #12, 40, 45, 62, 88, and 127 had the shortest intervals between LH surges within their group.
Hens #5, 26, 47, 66, 85, and 128 had intervals between LH surges closest to the mean within their group.
Hens #9, 32, 60, 83, 86, and 126 had the longest intervals between LH surges within their group.

Table 6.2. Mean interval between luteinizing hormone (LH) surges, mean duration of follicular development during 10 d of serial bleeding, and number of hierarchical follicles in each feeding group early and late in the reproductive period."
Table 6.3. Mean interval between luteinizing hormone (LH) surges during 10 d of serial bleeding and numbers of hierarchical follicles, identical sequence, multiple hierarchical follicles, and follicular development stages in each feeding group.¹

¹ Hens #4, 31, 45, 67, 88, and 127 had the greatest number of hierarchical follicles within their group.
Hens #5, 32, 60, 61, 93, and 135 had the number of hierarchical follicles closest to the mean within their group.
Hens #16, 26, 47, 63, 85, and 128 had the least number of hierarchical follicles within their group.
<table>
<thead>
<tr>
<th></th>
<th>Early FF</th>
<th></th>
<th>Late FF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#4</td>
<td>#5</td>
<td>#16</td>
<td>#67</td>
</tr>
<tr>
<td>Representative hens</td>
<td>#4</td>
<td>#5</td>
<td>#16</td>
<td>#67</td>
</tr>
<tr>
<td>LH surge interval (h)</td>
<td>31.3</td>
<td>32.3</td>
<td>33.4</td>
<td>37.0</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>11</td>
<td>8</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Identical sequence (n)</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Multiple hierarchy (n)</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Follicular development stage (n)</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Early RF</th>
<th></th>
<th>Late RF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#31</td>
<td>#32</td>
<td>#26</td>
<td>#88</td>
</tr>
<tr>
<td>LH surge interval (h)</td>
<td>26.1</td>
<td>38.0</td>
<td>30.4</td>
<td>28.8</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>11</td>
<td>9</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Identical sequence (n)</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Multiple hierarchy (n)</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Follicular development stage (n)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Early RR</th>
<th></th>
<th>Late RR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#45</td>
<td>#60</td>
<td>#47</td>
<td>#127</td>
</tr>
<tr>
<td>LH surge interval (h)</td>
<td>26.0</td>
<td>41.0</td>
<td>31.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Identical sequence (n)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Multiple hierarchy (n)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Follicular development stage (n)</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 6.4. Main effect means±SE for body, ovary and oviductal weights, number of atretic and hierarchical follicles, F\textsubscript{1} follicle weight, identical dye ring sequences, multiple hierarchical follicles, follicular development stages, follicular development, and rest period in broiler breeder hens early (Early) and late (Late) in the reproductive period and among feeding programs.

\textsuperscript{a-c} Means with no common superscript letter within rows and main effect are different ($P \leq 0.05$).

\textsuperscript{1}The hens were sampled early (Early) or late (Late) in the reproductive period. A group of hens was ad libitum-fed during growth and reproductive periods (FF). Second group of hens was restricted-fed during growth and was then switched to ad libitum-feeding after photostimulation (22 wk of age; RF). Third group of hens was restricted-fed during growth and reproductive period (RR).

\textsuperscript{2}Number of hierarchical follicles with identical dye ring sequence and number of dye rings.

\textsuperscript{3}Multiple hierarchical follicles were defined as the weight of 2 or more hierarchical follicles being less than 1 g different.

\textsuperscript{4}Hierarchical follicle(s) with the same number and sequence of dye rings were defined to be in the same follicular development stage.

\textsuperscript{5}Duration of follicular development (d) was estimated based on the number of dye rings in oviposited eggs.

\textsuperscript{6}Rest period was the number of days between the last dye ring deposition and oviposition.
<table>
<thead>
<tr>
<th>Experimental groups¹</th>
<th>Duration of the reproductive period</th>
<th>Feeding Program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Necropsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>4.35±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.93±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ovary (g)</td>
<td>12.45±0.72</td>
<td>13.70±0.64</td>
</tr>
<tr>
<td>Oviduct (g)</td>
<td>59.11±1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.50±1.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atretic follicle (n)</td>
<td>0.13±0.05</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>7.72±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.50±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F₁ (g)</td>
<td>14.7±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.52±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Identical sequence² (n)</td>
<td>1.78±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Multiple hierarchy³ (n)</td>
<td>1.27±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Follicle stages⁴ (n)</td>
<td>5.38±0.19</td>
<td>5.61±0.17</td>
</tr>
</tbody>
</table>

During 10 d of serial bleeding

<table>
<thead>
<tr>
<th></th>
<th>Follicular development⁵ (d)</th>
<th>Rest period⁶ (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.97±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.05±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.22±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 6.5. Interaction means±SE for body, ovary and oviductal weights, number of atretic and hierarchical follicles, F₁ follicle weight, identical dye ring sequences, multiple hierarchical follicles, follicular development stages, follicular development, and rest period in broiler breeder hens early (Early) and late (Late) in the reproductive period and among feeding programs.

<table>
<thead>
<tr>
<th>Feeding Program</th>
<th>Early (Early)</th>
<th>Late (Late)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a-c* Means with no common superscript letter within rows are different (\(P \leq 0.05\)).

1The hens were sampled early (Early) or late (Late) in the reproductive period. A group of hens was ad libitum-fed during growth and reproductive periods (FF). Second group of hens was restricted-fed during growth and was then switched to ad libitum-feeding after photostimulation (22 wk of age; RF). Third group of hens was restricted-fed during growth and reproductive period (RR).

2Number of hierarchical follicles with identical dye ring sequence and number of dye rings.

3Multiple hierarchical follicles were defined as the weight of 2 or more hierarchical follicles being less than 1 g different.

4Hierarchical follicle(s) with the same number and sequence of dye rings were defined to be in the same follicular development stage.

5Duration of follicular development (d) was estimated based on the number of dye rings in oviposited eggs.

6Rest period was the number of days between the last dye ring deposition and oviposition.
<table>
<thead>
<tr>
<th>Experimental groups&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Early-FF (n=10)</th>
<th>Early-RF (n=10)</th>
<th>Early-RR (n=13)</th>
<th>Late-FF (n=12)</th>
<th>Late-RF (n=14)</th>
<th>Late-RR (n=12)</th>
<th>( P= )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necropsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>5.11±0.14</td>
<td>4.17±0.14</td>
<td>3.74±0.12</td>
<td>5.46±0.12</td>
<td>4.75±0.12</td>
<td>4.55±0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Ovary (g)</td>
<td>14.62±1.29</td>
<td>11.29±1.23</td>
<td>11.23±1.07</td>
<td>17.60±1.12</td>
<td>11.92±1.12</td>
<td>11.59±1.12</td>
<td>0.64</td>
</tr>
<tr>
<td>Oviduct (g)</td>
<td>49.35±2.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.55±2.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.42±2.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.74±2.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.12±2.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.63±2.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Atretic follicle (n)</td>
<td>1.00±0.20</td>
<td>0.00±0.20</td>
<td>0.00±0.18</td>
<td>0.25±0.18</td>
<td>0.08±0.18</td>
<td>0.00±0.18</td>
<td>0.53</td>
</tr>
<tr>
<td>Hierarchical follicles (n)</td>
<td>8.13±0.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.10±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.92±0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.67±0.41&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.38±0.39&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.42±0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>( F_1 ) (g)</td>
<td>12.63±0.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.54±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.05±0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.79±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.11±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.50±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>Identical sequence&lt;sup&gt;2&lt;/sup&gt; (n)</td>
<td>2.13±0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.60±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.22&lt;sup&gt;de&lt;/sup&gt;</td>
<td>1.25±0.23&lt;sup&gt;bed&lt;/sup&gt;</td>
<td>1.00±0.22&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.17±0.23&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
<tr>
<td>Multiple hierarchy&lt;sup&gt;3&lt;/sup&gt; (n)</td>
<td>2.00±0.33</td>
<td>1.50±0.30</td>
<td>0.15±0.26</td>
<td>1.08±0.27</td>
<td>0.57±0.25</td>
<td>0.00±0.27</td>
<td>0.21</td>
</tr>
<tr>
<td>Follicle stages&lt;sup&gt;4&lt;/sup&gt; (n)</td>
<td>4.88±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00±0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.38±0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.25±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.38±0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.25±0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
</tbody>
</table>

During 10 d of serial bleeding

<p>| Follicular development&lt;sup&gt;5&lt;/sup&gt; (d) | 9.13±0.21 | 9.12±0.19 | 8.72±0.17 | 9.83±0.17 | 10.20±0.17 | 10.14±0.17 | 0.16 |
| Rest period&lt;sup&gt;6&lt;/sup&gt; (d) | 1.25±0.14 | 1.22±0.12 | 1.19±0.11 | 1.66±0.11 | 1.43±0.11 | 1.23±0.11 | 0.29 |</p>
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>r=0.49</td>
<td>r=0.06</td>
<td>r=0.22</td>
<td>r=0.29</td>
<td>r=0.20</td>
<td>r=0.20</td>
<td>r=0.37</td>
<td>r=0.07</td>
<td>r=0.29</td>
<td>r=0.32</td>
<td>r=0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P=0.00</td>
<td>P=0.64</td>
<td>P=0.07</td>
<td>P=0.02</td>
<td>P=0.11</td>
<td>P=0.09</td>
<td>P=0.00</td>
<td>P=0.55</td>
<td>P=0.02</td>
<td>P=0.01</td>
<td>P=0.01</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>--</td>
<td>r=0.09</td>
<td>r=0.13</td>
<td>r=0.22</td>
<td>r=0.17</td>
<td>r=0.16</td>
<td>r=0.14</td>
<td>r=0.09</td>
<td>r=0.08</td>
<td>r=0.08</td>
<td>r=0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P=0.47</td>
<td>P=0.29</td>
<td>P=0.73</td>
<td>P=0.17</td>
<td>P=0.18</td>
<td>P=0.24</td>
<td>P=0.45</td>
<td>P=0.53</td>
<td>P=0.49</td>
<td>P=0.62</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.28</td>
<td>r=0.09</td>
<td>r=0.29</td>
<td>r=0.12</td>
<td>r=0.05</td>
<td>r=0.24</td>
<td>r=0.14</td>
<td>r=0.13</td>
<td>r=0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.02</td>
<td>P=0.46</td>
<td>P=0.02</td>
<td>P=0.31</td>
<td>P=0.69</td>
<td>P=0.05</td>
<td>P=0.25</td>
<td>P=0.28</td>
<td>P=0.10</td>
</tr>
<tr>
<td>4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.19</td>
<td>r=-0.16</td>
<td>r=-0.11</td>
<td>r=0.24</td>
<td>r=-0.31</td>
<td>r=-0.19</td>
<td>r=0.10</td>
<td>r=-0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.12</td>
<td>P=0.20</td>
<td>P=0.36</td>
<td>P=0.05</td>
<td>P=0.01</td>
<td>P=0.11</td>
<td>P=0.40</td>
<td>P=0.17</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.11</td>
<td>r=0.81</td>
<td>r=0.41</td>
<td>r=0.50</td>
<td>r=0.11</td>
<td>r=0.11</td>
<td>r=0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.36</td>
<td>P=0.00</td>
<td>P=0.00</td>
<td>P=0.38</td>
<td>P=0.38</td>
<td>P=0.38</td>
<td>P=0.00</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.31</td>
<td>r=-0.37</td>
<td>r=0.36</td>
<td>r=0.57</td>
<td>r=0.18</td>
<td>r=0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.01</td>
<td>P=0.00</td>
<td>P=0.00</td>
<td>P=0.14</td>
<td>P=0.00</td>
<td>P=0.00</td>
</tr>
<tr>
<td>7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.51</td>
<td>r=0.13</td>
<td>r=-0.06</td>
<td>r=0.09</td>
<td>r=0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.00</td>
<td>P=0.30</td>
<td>P=0.62</td>
<td>P=0.46</td>
<td>P=0.00</td>
</tr>
<tr>
<td>8</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=-0.02</td>
<td>r=0.28</td>
<td>r=0.00</td>
<td>r=0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.85</td>
<td>P=0.02</td>
<td>P=1.00</td>
<td>P=0.01</td>
</tr>
<tr>
<td>9</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.17</td>
<td>r=0.23</td>
<td>r=0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.15</td>
<td>P=0.06</td>
<td>P=0.45</td>
</tr>
<tr>
<td>10</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.36</td>
<td>r=0.67</td>
<td>P=0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.00</td>
</tr>
<tr>
<td>11</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>r=0.15</td>
<td>P=0.21</td>
</tr>
<tr>
<td>12</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

1=body weight; 2=ovary weight; 3=oviductal weight; 4=number of atretic follicles; 5=number of hierarchical follicles; 6=F1 follicle weight; 7=number of identical sequences; 8=number of multiple hierarchical follicles; 9=number of follicular development stages; 10) duration of follicular development (d); 11=rest period (d); and 12=duration of the reproductive period (wk)

Table 6.6. Correlations (r) and statistical probability (P) in broiler breeder hens.
Fig 6.1  Dye ring sequence patterns produced by daily feeding of either Sudan IV (R) or Sudan Black B (B) to broiler breeder hens. The dye ring sequences (from the center of the yolk to the periphery) of daily dye feeding were; a) no dye-BRBRBRBBR, and (b) BRBRRBRRBRB, and the duration of follicular development was 11 d.
Figure 6.2. The relationship between duration of follicular development and oviposition during 10-d periods. Data from representative hens of each feeding program early and late in the reproductive period are presented at the top of each panel. Data from hens with shortest duration of follicular development are presented on left side, from hens with the medium duration presented in the center, and from hens with the longest duration on the right side. The closed circles represent the day of oviposition. Two open circles at the same d in the Early-FF hen #5 (6.2a) represent that this hen laid 2 soft shelled eggs in 1 h and the 2 ova had identical dye ring sequences and number of dye rings. The Late-RR hen #127 had a broken oviposited egg on d 4. No dye ring pattern for this yolk is shown in the chart. The sequence of dye rings is from the center (bottom) to the periphery (top) for each ovum. For example, the Early-FF hen #12 laid an egg at the first d during the trial, with the sequence from center to periphery of RBRBBRBR. The pattern of dye ring sequences for duration of ovarian follicular development, and weight of each hierarchical follicle at necropsy are presented in the bottom portion of each panel. The dye ring sequences presented are from the periphery (bottom) to center (top) for each ovarian hierarchical follicle. The hierarchical follicles with the same number and sequence of dye rings are defined as having the same follicular development stage (i.e. Early-FF hen #4). A difference in weight of hierarchical follicles of less than 1 g was defined as a multiple (double or triple) hierarchical follicle set. A. Early-FF hens. B. Early-RF hens. C. Early-RR hens. D. Late-FF hens. E. Late-RF hens. F. Late-RR hens.
Fig 6.2: Continued

B

B (Continued)
Fig 6.2: Continued
Fig 6.2: Continued

D (Continued)
Fig 6.2: Continued

F (Continued)


Bornstein, S., S. Hurwitz, and Y. Lev, 1979. The amino acid and energy requirements of 243


fatness on plasma leptin concentration assessed by a specific RIA in sheep.  J. Endocrinol. 165:519-526.


Duplaix, M., J. Williams, and P. Mongin, 1981.  Effects of an intermittent lighting schedule on the time of egg-laying and the levels of luteinizing hormone, progesterone and corticosterone in the plasma of the domestic hen.  J. Endocrinol. 91:375–383.


Etches, R.J., and K.W. Cheng, 1981. Changes in the plasma concentrations of luteinizing hormone, progesterone, oestradiol and testosterone and in the binding of follicle-stimulating hormone to the theca of follicles during the ovulatory cycle of the hen (Gallus domesticus). J. Endocrinol. 91:11-22.


Gage, S.H., and S.P. Gage, 1908. Sudan III. Deposited in the egg and transmitted to the 250


metabolic perturbation. J. Neuroendocrinol. 16:244-255.


Hocking, P.M., D. Waddington, M.A. Walker, and A.B. Gilbert, 1989. Control of the


Liu, H.K., and W.L. Bacon, 2004b. Changes in egg production induced by progesterone injection in broiler breeder hens. submitted


Luther, L.W., W.W. Abbot and J.R. Couch, 1976. Low lysine, low protein and
skip-a-day restriction of summer and winter reared broiler breeders pullets. Poult. Sci. 55:2240-2247.


McMurtry, J.P., W. Tsark, L. Cogburn, R. Rosebrough, and D. Brocht, 1996. Metabolic responses of the turkey hen (Meleagris gallopavo) to an intravenous injection of


National Research Council (NRC), 1994. Nutrition requirements of poult..  9th rev. ed. 263


265


two multiple blood sampling regimens using an indwelling vascular access device for investigations of the hen’s ovulatory cycle and calcium metabolism. Poult. Sci. 72:172-184.


