The role of feeding regimens in regulating metabolism of sexually mature broiler breeders

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ABSTRACT A trial was conducted to determine the effects of different rearing feed regimens on plasma hormone and metabolite levels and hepatic lipid metabolism and gene expression on sexually mature broiler breeders. Cobb 500 birds were divided into 2 groups at 4 wk and fed either an everyday (ED) or skip-a-day (SKP) regimen. At 24 wk of age, all birds were switched over to an ED regimen. At 26.4 wk, breeder hens were randomly selected and killed at intervals after feeding. Livers were sampled from 4 hens at 4-h intervals for 24 h for a total of 28 samples per treatment. Blood was sampled from 4 hens per sampling time; sampling times were 0, 30, and 60 min and 2 and 4 h after feeding and then every 4 h up to 24 h for a total of 36 samples per treatment. Main feeding regimen, time, and interaction effects were analyzed. Significant interaction effects were found between time and feeding regimen for acetyl-coenzyme A carboxylase and malic enzyme mRNA expression. The peak for acetyl-coenzyme A carboxylase expression was higher in ED-reared birds, whereas the peak for malic enzyme expression was higher in SKP-reared birds. Overall, plasma levels of insulin-like growth factor-II were higher in SKP-reared birds. Overall, plasma corticosterone levels were also higher in SKP-reared birds and significant interaction effects between time and feeding regimen were seen. The expression of apolipoprotein A1 was significantly higher in ED-reared birds: significant interaction effects were also noted. Other researchers also found some of the differences observed in the present study in 16-wk-old pullets. In summary, different feeding regimens alter metabolic responses, some of which carry over into sexual maturity.

Key words: broiler breeder, metabolic memory, rearing regimen, liver, fat metabolism

INTRODUCTION

Feed restriction regimens are a common tool used in the broiler breeder industry to improve egg production, delay sexual maturity, reduce BW, and increase livability. The effects of overfeeding include the incidence of double hierarchies and multiple ovulations due to accelerated ovarian follicular development (Robinson et al., 1993; Chen et al., 2006). The method of feed restriction varies, but the 2 common regimens are an everyday (ED) system and a skip-a-day (SKP) system. The SKP regimen is typically favored due to its ability to improve flock uniformity in scenarios in which feed space is limited (Cobb-Vantress, 2005). Feed restriction is usually initiated between 18 and 24 d of age and maintained until light stimulation, first egg, or 5% egg production.

The metabolic consequences of the different restriction feeding regimens have only recently been elucidated (deBeer et al., 2008). Both ED and SKP involve a period of fasting, differing only in length. Fasting is known to exert many metabolic changes, from anabolism to catabolism and lipogenesis to lipolysis. Levels of 3,3′,5-triiodothyronine (T3), insulin, and insulin-like growth factor-I (IGF-I) decrease during fasting, whereas plasma levels of glucocorticoid, insulin-like growth factor-II (IGF-II), and growth hormone increase (Decuypere and Kuhn, 1984; Buyse et al., 2000; Kita et al., 2002). Feed restriction can lead to chronic stress in chickens and an accompanying increase in plasma corticosterone (Nir et al., 1975).

Broiler breeder pullets on a SKP regimen had lower levels of IGF-I and leptin and higher levels of T3 and plasma corticosterone. Similarly, expression of acetylcoenzyme A carboxylase (ACC), fatty acid synthase (FAS), and malic enzyme were elevated in SKP-fed pullets with greater overall variability (deBeer et al., 2008). Programs such as SKP have been found to be
less efficient than ED programs (deBeer and Coon, 2007) due to the need to deposit and remobilize nutrients during the fasting period.

The concepts of metabolic memory and metabolic programming are not new. A large amount of evidence has arisen for the programming of body composition by early growth and nutrition (Wells et al., 2007). In such a scenario, adaptation to a nutritional stress alters, sometimes permanently, the metabolism of an animal and continues in the absence of the stress. Metabolic abnormalities such as obesity, glucose intolerance, and insulin resistance have been linked to early malnutrition in human and rats (Ihnat et al., 2007). Early feed restriction in broilers has been shown to alter growth performance and lipid metabolism even after restoration of ad libitum feeding (Zhan et al., 2007). This study attempts to determine whether the metabolic patterns established during the growing phase of broiler breeder hens (deBeer et al., 2008) remain at the onset of sexual maturity.

**MATERIALS AND METHODS**

**Bird Use**

All procedures were carried out in accordance with Animal Use Protocol No. 06012 for the experiment, which was approved by the University of Arkansas Institutional Animal Care and Use Committee. A flock of 480 Cobb 500 pullets was reared in floor pens from 1 d of age utilizing the Cobb 500 Breeder Management Guide (Cobb-Vantress, 2005) as a reference for all management conditions. The compositions of the diets used throughout the experiment are shown in Table 1. Diets did not differ between treatments. The starter diet was fed from 0 to 4 wk of age, the grower diet was fed from 4 until 20 wk, the prebreeder diet was fed from 20 until 25 wk, and the breeder I diet was fed from 25 wk until termination of the feeding study at 26.4 wk. The flock was fed ad libitum for the first 2 wk. From 2 to 4 wk, all birds were fed restricted amounts of feed every day. At 4 wk of age, feed restriction treatments were implemented. Two hundred forty pullets were fed restricted amounts of feed every day from 4 to 21 wk of age. Feed allocation was based on breeder-recommended guidelines to reach target BW (Table 2). Another 240 pullets were fed every other day. Total feed allocation did not differ between the 2 treatments. For example, if the ED group received 50 g per bird, the SKF group would receive 100 g per bird every other day. Breeder pullets were weighed weekly in groups to adjust feed allocation to ensure that target BW was met. Birds were weighed weekly by pen to adjust feed allocation to ensure target BW was met.

At 21 wk, 211 birds representing each of the rearing regimens were randomly selected and transferred to a production house and individually caged. Cages (47 cm high, 30.5 cm wide, 47 cm deep) were each equipped

<table>
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<tr>
<th>Table 1. Composition (%) of diets and nutrient contents</th>
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<td>Ingredient</td>
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<td>Wheat middlings</td>
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<td>Dicalcium phosphate</td>
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<td>Ground limestone</td>
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<td>l-Lysine HCl</td>
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1Mold inhibitor (Anitox Corp., Lawrenceville, GA).
3Mineral mix provided the following per kilogram of complete diet: Cu, 18 mg; I, 1.1 mg; Fe, 80 mg; Mn, 150 mg; Zn, 125 mg; and Se, 0.25 mg.
4Vitamin mix provided the following per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D₃, 3,000 IU; vitamin E, 100 IU; vitamin K₃, 3 mg; vitamin B₁₂, 0.03 mg; riboflavin, 8 mg; niacin, 60 mg; pantothenic acid, 18 mg; folic acid, 1 mg; pyridoxine HCl, 6 mg; thiamine HCl, 3 mg; and biotin, 0.2 mg.
5Corrected to 90% DM.
with an individual feeder and nipple drinker. Birds were fed individually and provided with free access to water at all times. At 24 wk, all birds were put on an ED feeding system. At 26.4 wk, which corresponded to 10 d after 5% production, 28 sexually mature breeders from each rearing regimen were fed 125 g of the breeder diet and 4 hens were killed via CO2 asphyxiation at 4-h intervals for 24 h. Livers from killed hens were excised and immediately snap-frozen in liquid N for analysis of gene expression, enzyme activities, lipid, and glyco- gen content. Blood samples were collected from 4 birds from each feeding regimen immediately before feeding and then at intervals after feeding. The sampling intervals were 0 min, 30 min, 60 min, 2 h, and 4 h after feeding and then every 4 h up to 24 h. Blood samples (5 mL) were collected from each bird by cardiac puncture using EDTA as anticoagulant in a 10-mL syringe fitted with a 38.1-mm, 20-gauge needle. The blood samples were centrifuged in a TJ-6 centrifuge (Beckman Coulter Inc., Fullerton, CA) for 10 min at 1,000 × g and plasma was stored at −20°C for further analysis of leptin, glucagon, insulin, T3, T4, corticosterone, and leptin as described by deBeer et al. (2008). The intraassay CV was 2.9% for insulin, 3.1% for glucagon, 3.7% for IGF-I, 4.1% for IGF-II, 6.6% for T3, 5.4% for T4, 5.2% for corticosterone, and 6.7% for leptin. All samples were analyzed within 1 assay to avoid interassay variations.

**Statistical Analysis**

Data analysis was performed using JMP IN 7.0.1 statistical analysis software (SAS Institute Inc., Cary, NC). Birds were assigned to treatments in a completely random manner. Data were analyzed using the GLM. The statistical model included terms for feeding regimen, time after feeding, and the interaction of the two. Data are presented as mean ± SEM and consist of 4 replicates per observation. All statements of significance are based on testing at \( P \leq 0.05 \).

**RESULTS**

**Liver Weights and Glycogen and Lipid Contents**

There were no overall mean differences by feeding regimen, time, or interaction for relative liver weight, total liver fat, and liver fat percentage (Figure 1). Liver Part of the remaining liver tissues were homogenized (1:10, wt/vol) in 100 mM HEPES (pH 7.5) and 3.3 mM β-mercaptoethanol and centrifuged at 12,000 × g for 30 min (Rosebrough and Steele, 1985). The supernatant fractions were kept at −80°C until analyzed for the activities of malic enzyme (EC 1.1.1.40) and ICDH (EC 1.1.1.42). Malic enzyme activity was determined by a modification of the method of Hu and Lardy (1969) as described by deBeer et al. (2008). Isocitrate:NADP+ oxidoreductase (decarboxylating) activity was determined by a modification of the method of Cleland et al. (1969) as described by deBeer et al. (2008).

**Liver Glycogen and Lipid Content**

Livers were analyzed for glycogen with amyloglucosidase digestion (Keppler and Decker, 1974) followed by glucose residue analysis (Hill and Kessler, 1961). The remaining portions of all excised livers were frozen at −20°C until further processing. Liver portions were subsequently lyophilized in a Genesis SQ 12 EL Freeze Drier (The Virtis Company, Gardiner, NY) to determine the DM content. Lyophilized samples were ground before further analysis. Total liver fat was analyzed according to method 920.39 (AOAC, 1990). Liver fat was determined on a DM basis by multiplying the dry liver weight by the calculated fat in the dry liver sample.

**Gene Expression and Enzyme Activity**

Single-step reverse transcription-PCR on a real-time basis was used to quantify mRNA expression of FAS, malic enzyme, ACC, aspartate aminotransferase (AAT), isocitrate dehydrogenase (ICDH), apolipoprotein A1 (apo A1), apolipoprotein B (apo B), apolipoprotein very low density lipoprotein-11 (apo VLDL-II), fatty acid binding proteins (FABP), and β-actin as described by deBeer et al. (2008).
glycogen had almost identical response curves between treatments; mean values of SKP-reared birds were above ED-reared birds at all time points (Figure 1A). The relative liver weight of SKP birds rose immediately after feeding to a peak at h 12 before declining (Figure 1B). Relative liver weight for ED-reared birds fell immediately after feeding to a minimum at h 8 and steadily rose to prefeeding levels. Both treatments had similar total liver fat patterns (Figure 1C). Total liver fat rose after feeding to a peak at h 12, with SKP-reared breeders having a higher peak. The percentage of liver fat had similar results to total liver fat (Figure 1D). Both treatments increased liver fat percentage with a peak at h 12 with the SKP regimen having a higher peak.

**Hepatic Enzyme Activity**

No differences were noted in the overall means of ICDH and malic enzyme activity by feeding regimen or time, but a significant interaction effect was noted in ICDH activity (Figure 2). Isocitrate dehydrogenase activity in SKP-reared hens did not immediately increase after feeding, only reaching a peak at h 20 (Figure 2A). Isocitrate dehydrogenase activity in the ED-reared hens rose initially before dropping to a minimum at h 8. Malic enzyme activity fell immediately after feeding for both treatments and reached peaks at h 8 (Figure 2B). The peak was higher in the ED-reared hens.

**Hepatic Gene Expression**

Expression of ACC, AAT, FAS, ICDH, and malic enzyme did not differ by feeding regimen; however, significant time effects were seen (Figure 3). Expression of ACC in ED-reared hens rose dramatically immediately after feeding and fluctuated before dropping to prefeeding levels at h 24 (Figure 3A). Expression of ACC in SKP-reared birds showed a steady increase to a peak at h 20 before decreasing to below prefeeding levels at h 24. Significant interaction effects were noted in ACC expression ($P = 0.0130$). Expression of AAT in ED-reared hens showed a steady increase after feeding to a peak at h 12 (Figure 3B). Expression of AAT in SKP-reared hens fluctuated until peaking at h 16 and fell thereafter. Expression of FAS rose after feeding for both feeding regimens (Figure 3C). Skip-a-day-reared hens peaked at h 8 with approximately a 6-fold increase from prefeeding levels. Everyday reared hens peaked at h 12 before decreasing to prefeeding levels. Expression of ICDH for hens from both feeding regimens steadily rose throughout the 24-h period; however, the SKP-reared hens showed a spike at h 16 (Figure 3D). Ex-
pression of malic enzyme rose immediately after feeding to a peak at h 4 for ED-reared hens and h 8 for SKP-reared hens (Figure 3E). Both fell to prefeeding levels by h 24. Significant interaction effects were also seen for malic enzyme expression ($P = 0.0005$).

**Plasma Hormones**

Overall plasma glucagon levels did not differ between feeding regimens (Figure 4A). Significant time effects were seen but no interaction effects. Plasma glucagon fell immediately after feeding at h 1 for the ED-reared birds and h 4 for SKP-reared birds before returning to near prefeeding levels. Overall insulin levels did not differ between feeding regimens; however, significant time effects were seen (Figure 4B). Immediately after feeding, plasma insulin levels spiked within the first 0.5 h and fell thereafter. Plasma IGF-I levels did not differ overall (Figure 4C). Skip-a-day-reared hens had higher levels of overall plasma IGF-II, but no time differences were noted (Figure 4D). No feeding regimen differences were noted in plasma T₃ and T₄ levels. Significant time effects were seen in T₃ but not T₄ (Figure 4E and F). Plasma leptin levels fluctuated throughout the sampling period with an overall time effect but nonsignificant overall differences between the 2 feeding regimens (Figure 4H). Skip-a-day-reared hens had higher levels of corticosterone ($P = 0.0068$). Overall time differences and interaction effects were noted in corticosterone levels as well (Figure 4G).

**Lipoproteins**

No overall mean differences due to feeding regimen were seen for mRNA expression of apo B ($P = 0.1647$), apo VLDL-II ($P = 0.3755$), or FABP ($P = 0.4294$; Figure 5). However, ED-reared hens had higher levels of expression of apo A1 mRNA ($P = 0.0016$). Time and interaction effects on apo A1 expression were noted. The apo A1 mRNA expression at the prefeeding time was over 6-fold higher in the ED-reared hens (Figure 5A). After this time point, except for a small spike at h 20 in the ED-reared hens, apo A1 mRNA expression between the 2 feeding regimens mirrored each other throughout the 24-h period. No significant feeding regimen, time, or interaction effects were noted for apo B mRNA expression. A decrease in the expression of apo B levels in ED-reared hens was seen immediately after feeding, reaching a minimum at h 12 (Figure 5B). An increase in apo B levels was seen in SKP-reared hens at h 12, after which apo B levels mirrored each other between feeding regimens. No significant feeding regimen, time, or interaction effects were noted for apo VLDL-II mRNA expression. The apo VLDL-II in ED-reared hens followed similar patterns to that of apo B: apo VLDL-II reached a minimum at h 8 instead of h 12 as in apo B (Figure 5C). The SKP-reared hens did not share patterns between apo VLDL-II and apo B. Our findings show that apo VLDL-II fell to a nadir at h 4 before rising at h 12. Significant time effects were noted for FABP mRNA expression. No significant feeding regimen differences in FABP mRNA expression were seen. Birds on both feeding regimens had similar response curves throughout the 24-h sampling period (Figure 5D).

**DISCUSSION**

After a meal, glycolysis provides substrate in the form of acetyl-coenzyme A for de novo fatty acid synthesis. Increased lipogenic capacity in feeding regimens in which carbohydrate supply is more abundant would, therefore, be expected. In chickens, the liver is the major site of lipogenesis. Ad libitum feeding in chicks was found to maintain a smaller maximum liver size com-
pared with meal-fed chicks (Muiruri et al., 1975). Skip-a-day feeding was found to increase overall relative liver weight in 16-wk-old pullets compared with ED feeding (de Beer et al., 2007). In 26-wk-old birds, relative liver weight of SKP-reared birds was not significantly higher than ED-reared hens \( (P = 0.1015) \). The findings by Muiruri et al. (1975) and de Beer et al. (2007) seem to indicate that consistency in nutrient supply has a major influence on maximum liver size and the fluctuations in liver size mirror fluctuations in nutrient supply. The research reported herein show that SKP-reared hens had more variable relative liver weight patterns, patterns that closely mimicked those found at 16 wk. However, ED-reared hens had fluctuations in relative liver weight that were more consistent with the ED feeding regimen.

Figure 3. Hepatic expression (relative to β-actin) of the genes coding for A) acetyl-coenzyme A carboxylase (ACC), B) aspartate amino transferase (AAT), C) fatty acid synthase (FAS), D) isocitrate dehydrogenase (ICDH), and E) malic enzyme, as determined using real-time reverse transcription-PCR, for hens reared on an everyday (ED) and skip-a-day (SKP) regimen during a single feeding cycle (24 h) at 26.4 wk; hens were fed a 125-g meal. Data are means of 4 observations at each time point. Dark periods are indicated by dark bars on the timeline.
Figure 4. Plasma concentrations of A) glucagon (pg/mL), B) insulin (ng/mL), C) insulin-like growth factor-I (IGF-I, ng/mL), D) insulin-like growth factor-II (IGF-II, ng/mL), E) triiodothyronine (pg/mL), F) thyroxine (pg/mL), G) corticosterone (ng/mL), and H) leptin (ng/mL) in everyday (ED)- and skip-a-day (SKP)-reared hens during a single feeding cycle (24 h) at 26.4 wk; hens were fed a 125-g meal. Data are means of 4 observations at each time point. Dark periods are indicated by dark bars on the timeline.
Relative liver weight in breeder hens fell immediately after feeding, contrary to findings by de Beer et al. (2007) for breeder pullets. To establish that metabolic memory is being observed, lipogenic characteristics should remain elevated in SKP sexually mature breeders and the relationship of hormone and lipogenic components for both regimens should be comparable to those found at 16 wk. When these 2 conditions are not met, 2 possible explanations are possible. The birds have adapted to the new physiological state (i.e., feeding regimen, sexual maturity) or the results seen at 16 wk more closely resembled patterns in mature breeders rather than pullets. It has been reported that hens on an ED feeding regimen reached sexual maturity earlier than did hens on a SKP feeding regimen (de Beer and Coon, 2007).

Increases in liver weight have previously been attributed to deposition of glycogen in the liver (Muiruri et al., 1975), but further evidence shows a significant contribution by lipid as well (de Beer et al., 2007). No significant differences were determined between SKP- and ED-reared hens for total glycogen and liver fat; however, the overall means of glycogen for SKP-reared hens were greater than those of ED-reared hens at all time points. Both feeding regimens had similar patterns at 26 wk and closely mimicked the pattern at 16 wk, the only distinction being that the patterns observed at 26 wk were condensed to 24 h rather than 36 to 48 h at 16 wk. Early restricted feeding during the first 21 d and subsequent ad libitum feeding in broilers resulted in decreased carcass yield, abdominal fat, and breast muscle at 21 d (Zhan et al., 2007).

Acetyl-coenzyme A carboxylase catalyzes the rate-limiting step of lipogenesis. It is controlled in the short-term by covalent modification and allosteric control by citrate. Long-term control is mediated by transcriptional mechanisms through insulin, glucagon, T3, and glucose (Hillgartner et al., 1995, 1996). Overall expression of ACC in SKP-reared hens (118.67) was not significantly different (P = 0.0754) than ED-reared hens (115.20). The pattern of expression in SKP-reared hens was more consistent and was similar to results seen at 16 wk. The ED-reared hens showed large surges in ACC expression immediately after feeding and then again at h 12; dissimilar to results in pullets. The surges seen in both treatments may have to do with the timing of egg formation and synthesis of yolk. Under a scenario in which SKP-reared hens are in an enhanced lipogenic state, they would already have the capacity to handle

![Figure 5](https://example.com/figure5.png)

Figure 5. Hepatic expression (relative to β-actin) of the genes coding for A) apolipoprotein A1 (apo A1), B) apolipoprotein B (apo B), C) apolipoprotein very low density lipoprotein-II (apo VLDL-II), and D) fatty acid binding proteins (FABP) as determined using real-time reverse transcription-PCR for hens reared in an everyday (ED) and skip-a-day (SKP) regimen during a single feeding cycle (24 h) at 26.4 wk. Data are means of 4 observations at each time point. Dark periods are indicated by dark bars on the timeline.
the additional burden of lipid synthesis for egg deposition. On the other hand, ED-reared hens would need to switch over to a lipogenic state for egg formation. The malic enzyme reaction is crucial to the production of reducing equivalents (NADPH) for fatty acid synthesis. Hillgartner et al. (1996) reported that dietary control of FAS and malic enzyme gene transcription is coordinated with that of ACC in chicken liver and suggested a common control mechanism for hepatic lipogenic enzyme genes in chickens. Lifelong elevation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and FAS due to a high-carbohydrate diet during the weaning period has been noted in rats (Hahn, 1984). It is unclear whether a large influx of nutrients creates the same response observed by Hahn in chickens. Malic enzyme was found to be elevated in feed-restricted birds (Richards et al., 2003) and in 16-wk-old pullets on a SKP feeding regimen (de Beer et al., 2008). The research reported herein does not conclusively show that malic enzyme expression in SKP-reared hens remains elevated, although significant interaction effects and a higher peak in SKP-reared hens suggests that the previous findings may still hold true in mature breeder hens. Fatty acid synthase expression was not significantly higher in SKP-reared birds.

Aspartate aminotransferase and ICDH gene expressions may be indicative of the degree of glucose production from precursors (gluconeogenesis). Both enzymes are involved in the tricarboxylic acid cycle and the biosynthesis of amino acids. Increasing dietary protein has been found to reduce ACC activity and lipogenesis; it has been suggested that competition for citrate between ACC and aconitase-ICDH may be responsible for these results (Rosebrough et al., 2002). de Beer et al. (2008) found that expression of AAT and ICDH did not differ for ED-fed pullets throughout the feeding cycle. On the other hand, SKP-fed pullets had decreased levels of AAT and ICDH immediately after feeding. Sexually mature hens showed an increase in AAT and ICDH expression in the latter part of the 24-h feed cycle regardless of rearing regimen, corresponding to times of fasting. These results suggest that gluconeogenesis in mature hens is not affected by rearing regimen. It has also been suggested that ICDH may serve a role in producing reducing equivalents (NADPH) for lipogenesis. Several authors agree that this role is at best limited (Lee et al., 2001; Richards et al., 2003; de Beer et al., 2007). Zhan et al. (2007) found that restricted feeding during the first 21 d and subsequent ad libitum feeding in broilers resulted in numerical increases in the malic enzyme and ICDH lipogenic enzymes at 21 d. By 63 d, abdominal fat was higher in early restricted fed birds, and lipogenic enzymes had become significantly elevated. Early restricted feeding in the study by Zhan et al. (2007) upregulated lipogenesis similar to a fasting-refeeding cycle (Rosebrough and Steele, 1985). Even after feed restriction has been removed, the physiological changes remain.

The coordination of hormones during the transition into sexual maturity and its consequences on production are of great interest. Plasma glucagon patterns were similar between rearing regimens; overall levels were not significantly higher \((P = 0.0790)\) by the SKP regimen \((319.78)\) compared with the ED regimen \((278.18)\). A spike in glucagon at h 1 in the SKP-reared hens appears to correspond to a large decrease in insulin level at around this time. Insulin levels peaked for both rearing regimens within 30 min and slowly declined over time. The peak seen in the SKP-reared hens is higher than in the ED regimen but to a smaller degree than was observed at 16 wk (de Beer et al., 2008). The hormonal response to a set amount of feed was of greater magnitude in SKP-reared hens and was lipogenic in nature. The overall levels of IGF-II were higher in SKP-reared hens. No differences were noted in overall IGF-I levels at 26 wk. de Beer et al. (2008) showed different results at 16 wk. Sixteen-week-old pullets showed no difference in IGF-II levels; however, the ED pullets showed a significantly higher IGF-I level. Kita et al. (2002) showed that fasted birds had lower levels of IGF-I and higher levels of IGF-II. Elements of these findings can be found in the results by de Beer (lower IGF-I in SKP-reared hens) and the study presented here (higher IGF-II in SKP-reared hens). Overall levels of circulating T3 in sexually mature breeders did not differ based on rearing regimen, although response patterns between rearing regimens were similar. de Beer et al. (2008) showed much the same results at 16 wk. However, the patterns were slightly altered at 26 wk from what was seen at 16 wk. A small decline in T3 was observed immediately after feeding in 26-wk-old hens, whereas 16-wk-old pullets increased immediately after feeding. The reason for this observation is unclear. Increased lipogenesis has been demonstrated in previously fasted-refed birds (Rosebrough and Steele, 1985) and was partially ascribed to increased circulating T3 levels. The research reported herein further supports the claim that T3 levels are linked to lipogenesis and the fasting period. Thyroxine levels did not fluctuate significantly throughout the feeding cycle for either rearing regimen. The conversion of T4 to active T3 can be regulated by feeding regimens (Rosebrough and Steele, 1985). It has been postulated that the observed increase in T3 may have to do more with the prevention of degradation of T3 rather than an increase in conversion of T4 to T3 (Buysse et al., 2000; Swennen et al., 2005).

Corticosterone is a stress hormone that was shown to be significantly elevated in pullets on a SKP feeding regimen (de Beer et al., 2008). This was attributed to the competition that arises for a limited amount of feed. The elevation of overall plasma corticosterone in SKP-fed pullets at 16 wks was also seen in SKP-reared birds at 26 wk. Despite a normalized feeding schedule, SKP-reared breeder hens were still showing increased corticosterone with a peak over 3-fold greater than ED-reared breeders. In pullets, spikes in corticosterone
were attributed to crop emptying and hunger. Corticosterone also functions in mediating other physiological functions including lipolysis and gluconeogenesis (Cahill, 1971; Olefsky, 1975; Amatruda et al., 1985). The lack of a spike for ED-reared hens and the overall lower levels suggest that SKP-reared hens have an altered metabolism that necessitates nutrient mobilization when one is not needed in ED-reared hens.

Although overall plasma leptin was not higher in SKP-reared hens ($P = 0.0549$), it was a reversal of the results obtained from pullets (deBeer et al., 2008). It has been surmised that leptin plays an important role in reproduction (Clarke and Henry, 1999; Caprio et al., 2001; Cassy et al., 2004). The transition into sexual maturity has not been completely explored in broiler breeders, but body composition appears to regulate the transition into sexual maturity. Adiposity and leptin secretion appear to be related because leptin signals the hypothalamus to suppress feed intake and increase energy expenditure. The reversal in leptin levels in breeder hens compared with breeder pullets for the 2 feeding regimens may reflect the link between body composition and sexual maturity. In established obesity, selective leptin resistance at the level of the hypothalamus has been proposed. Maternal or neonatal hyperleptinemia in the immediate postnatal period could program selective leptin resistance and therefore a propensity to both obesity and obesity-related hypertension (Taylor and Poston, 2007). The elevated levels of leptin in SKP-reared hens in the present study are consistent with elevated lipogenesis.

Apolipoprotein B100 is the major apolipoprotein of very low density lipoproteins in chickens, whereas apo A1 is associated with high-density lipoproteins. Laying hens use apo VLDL-II along with apo B in their assembly of a yolk-targeted very low density lipoprotein (Hermier, 1997; Richards et al., 2003). In the present study, no overall differences were noted in the expression of apo B mRNA and apo VLDL-II mRNA between rearing regimens. The patterns of apo B and apo VLDL-II mRNA expression are similar with both reaching minimum between 8 and 12 h. The SKP-reared breeders had a significantly higher expression of apo A1; apo A1 had a pattern that was opposite to that of apo B and apo VLDL-II. Richards et al. (2003) found that both restricted and ad libitum-fed breeder hens had elevated levels of apo B expression, apo VLDL-II expression, and FABP expression compared with breeder pullet levels, whereas, apo A1 expression was significantly diminished in hens compared with pullet levels. Only FABP showed differences between restricted and ad libitum-fed birds during the breeder phase.

In summary, SKP-reared hens show characteristics of increased lipogenesis compared with ED-reared hens, a pattern that was established during the pullet stage by the feeding regimen. The carryover of certain traits (corticosterone, malic enzyme, and ACC) may be due to early development in SKP birds. The adaptation to a shortened feeding cycle, yet still retaining similar patterns to the pullets, provides evidence of metabolic memory. The resulting lipogenic state may be detrimental at later stages of a breeder’s productive life. It has been reported that overfeeding hens can negatively affect egg production, alter plasma lipoprotein profiles, and in severe cases lead to fatty liver hemorrhage syndrome (Walzem et al., 1993, 1994). Further investigation is needed into the consequences of pullet-rearing regimens on breeder metabolic processes.

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