A twice-a-day feeding regimen optimizes performance in broiler breeder hens

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ABSTRACT To evaluate the effects of different methods of feed allocation on performance, plasma hormone concentration, and ovarian morphology, an experiment was conducted using 32 Cobb 500 broiler breeder hens. The experiment was started at 27 wk and lasted to 39 wk of age. Feeding regimens included: i) hens with an ad libitum feeding program, ii) hens that received their restricted feed once a day, iii) hens that received their restricted feed twice a day, and iv) hens that received their restricted feed 3 times a day. Each hen was assumed as an experimental unit and treatments were replicated 8 times. Daily egg production, BW, and egg and yolk weights were measured. Two blood samples were taken 3 and 6 h after the first feed allocation every 2 wk. Plasma samples were assayed for glucose, triacylglycerol (TAG), cholesterol, as well as leptin-like concentration, glucagon, triiodothyronine, thyroxine, progesterone, estradiol, and testosterone. Liver, abdominal fat pad, and ovary were collected at necropsy. Ovaries were weighed and follicles were characterized as large yellow follicles, small yellow follicles, and large white follicles. Results showed inferior egg production in ad libitum-fed birds along with high levels of plasma glucose, TAG, cholesterol, leptin-like concentration, and testosterone. Twice-a-day-fed birds produced more egg in the entire production period than once-a-day-fed birds. Better performance of twice-a-day-fed hens was associated with lower plasma glucose, TAG, and leptin-like concentration, whereas their estradiol and glucagon were higher than once-a-day-fed hens. Results obtained in our study suggest that allocation of restricted feed 2 times a day may alleviate or delay lipotoxicity development and improve reproductive performance in broiler breeder hens.

Key words: twice-a-day feeding, hyperleptinemia, lipotoxicity, broiler breeder

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INTRODUCTION

Genetic selection for growth parameters in meat-type chickens gives rise to a parent stock (broiler breeders) that tends to lack the ability to self-regulate feed intake. As such, their high body mass is associated with excessive fat deposition, lameness, and high mortality rates (often due to skeletal or cardiovascular disease, or both). To regulate weight gain, limit health risks, and also maintain high fertility, husbandry practices for the parent stock of broiler chickens encompass a high degree of feed restriction (Renema and Robinson, 2004). This restricted amount of feed is rapidly consumed; therefore, the hens fast for an extended period of time before their next feeding. Although feed restriction programs improve egg production of broiler breeders, these hens still have inferior total egg production compared with commercial laying hens. Morris and Nalbandov (1961) reported that a prolonged period of fasting reduced gonadotropin secretion and resulted in inferior egg production in fasted birds. Cave (1981) demonstrated that feeding broiler breeder hens 3 times a day increased egg production during the first 10 wk of production compared with hens fed once or twice a day. More recently, Spradley et al. (2008) reported that broiler breeders fed twice a day laid more and heavier eggs through 42 wk of age than those fed once a day. They also had better overall BW uniformity. Chen et al. (2006) reported that high glucose availability due to hyperphagia in broiler breeders could result in lipotoxicity and ovarian dysfunction. Lipotoxicity is associated with nonfunctional leptin signaling, excessive accumulation of triacylglycerol and fatty acids in nonadipose tissues, as well as altered circulating and tissue lipid profiles (Chen et al., 2006). Because increased feeding frequency is a common recommendation to patients with type 2 diabetes to prevent increased blood glucose (Wadhwa et al., 1973), we hypothesized that increased feeding frequency may improve broiler breeder egg production by decreasing plasma glucose and preventing lipotoxicity development. The objective of the present study was to determine whether differences in plasma concentrations of leptin-like concentration, glucagon, estradiol ($\mathbf{E_2}$), progesterone ($\mathbf{P_4}$), testosterone, triiodothyronine

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($\mathbf{T_3}$), thyroxine ($\mathbf{T_4}$), as well as glucose, triacylglycerol (**TAG**), and cholesterol in feed-restricted, feed-satiated, twice-a-day and 3-times-a-day-fed broiler breeder hens could be related to egg and ovarian indexes.

MATERIALS AND METHODS

Birds and Management

A total of 32 Cobb 500 broiler breeder pullets with similar BW (2,050 ± 40 g) were selected from a commercial flock (20 wk of age) and moved to individual research cages (0.6 × 0.4; 0.24 m^2/bird). During the rearing period, pullets were restricted on an everyday feeding program. Soft plastic wires were placed on cage floors to minimize bird's foot damages. Each cage was equipped with an individual feeder and a nipple waterer. Birds did not have any access to the feed of each other. Pullets were fed restricted amounts of feed to provide a standard BW according to the Cobb Breeder Management Guide (Cobb-Vantresss, 2005) until the initiation of the experimental period. Birds were fed a prelay diet from wk 21 to first egg production and a broiler breeder layer diet thereafter (2,700 kcal of AME_n and 15.20% CP). Photostimulation occurred at 22 wk of age by providing 16 h of light (lights on at 0700 h), and this photoperiod was maintained until experiment termination. The temperature in the house was kept at around 22°C.

At 27 wk of age (50% egg production), hens were selected on the basis of their egg production and BW and assigned to feeding regimens to ensure that the egg production and weight profile in each group were similar. The experiment was started at 27 wk and lasted to 39 wk of age. The following feeding regimens were included: i) hens with an ad libitum feeding program (**ALF**), ii) hens that received their restricted feed once a day (**R1D**), iii) hens that received their restricted feed twice a day (**R2D**), and iv) hens that received their restricted feed 3 times a day (**R3D**). Each feeding regimen was replicated 8 times and each hen accounted as a replicate. The amounts of restricted feed were calculated based on BW and egg production in R1D hens according to the Cobb Breeder Management Guide (Cobb-Vantress, 2005). The ALF hens received 50% more feed than their restricted counterparts. Based on daily observations, this amount of feed was enough to establish ad libitum feeding terms. Hens from all regimens received their feed on an everyday feeding program from 20 wk of age until the end of the experiment. The birds for ALF and R1D feeding treatments received all of their feed at 0730 h, whereas feed for the birds of the R2D and R3D feeding programs was split in 2 and 3 equal parts and was fed at 0730 and 1130 h (R2D feeding program) and 0730, 1130, and 1430 h (R3D feeding program), respectively. The birds in feeding regimens other than the ALF group received the same total amount of daily feed. Feed cleanup time (when the birds ceased eating once the food was offered) was measured on 3 consecutive days (d 29 to 31 of experiment). All of the hens were weighed at weekly intervals early in the morning before offering the feed.

Laying Performance

Eggs were manually collected 2 times per day. Actual daily egg production was calculated for every 2-wk period from daily egg counts. The numbers of abnormally double-yolk and soft-shell eggs were recorded daily. Eggs and yolk weights were measured during 3 consecutive days every week and then egg mean weight (EMW) and yolk mean weight (YMW) and also yolk fractional weight (YMW/EMW × 100) were calculated for every 2-wk period. Yolk weights were measured after gently rolling the yolk on the paper towel to remove adherent white.

Necropsy and Tissue Collection

At the end of the experimental period, 4 hens per feeding regimen were selected randomly and anesthetized for necropsy. Liver, abdominal fat pad, and ovaries were collected at necropsy. Weight of liver, ovaries, and abdominal fat pad were divided by 100 (BW/100) to estimate their fractional contribution. Ovaries were weighed (after removing hierarchical follicles) and follicles were classified into 3 groups: hierarchical follicles (large yellow follicles, **LYF**; >8 mm), small yellow follicles (2 to 8 mm), and large white follicles (**LWF**; 2 to 5 mm) according to the system devised by Gilbert et al. (1983).

Blood Sampling

To evaluate plasma hormones and metabolites, first bleeding was carried out before initiation of the experiment (27 wk of age) and subsequent bleedings were repeated every 2 wk. Two separate blood samples were taken in each bleeding at 1030 and 1330 h (3 and 6 h after offering first feed, respectively). Blood samples were collected in EDTA-coated tubes from the brachial vein of all hens per group. A different wing was used in each bleeding. Blood samples were immediately centrifuged at 2,000 × g for 15 min to collect blood plasma. Plasma samples were stored at −20°C pending for glucose, TAG, cholesterol, as well as leptin-like concentration, glucagon, T_3, T_4, P_4, E_2, and testosterone assays. Plasma samples obtained at 1030 h bleeding (3 h after feed allocation) were analyzed only for glucose.

Plasma Metabolites and Hormones

Plasma glucose, total TAG, and total cholesterol were determined enzymatically using an automated analyzer (Hitachi 902, Tokyo, Japan). Specific RIA was used to determine plasma hormone concentrations. All samples were analyzed within 1 assay to avoid interassay variations. Plasma leptin-like concentration was determined

by a multispecies leptin RIA kit (Linco Research Inc., CliniSciences, Montrouge, France) with an intraassay CV of 9.63% according to the recommendations of the manufacturer. The use of the kit for chicken leptin has previously been validated (Cassy et al., 2004). Plasma glucagon was determined using a commercial multispecies ELISA kit (028-02, Phoenix Pharmaceuticals, Burlingame, CA) with an intraassay CV of 11.06%. Plasma P_4 and E_2 were determined by commercial RIA kits (ICN Biochemicals, Cleveland, OH) with intraassay CV of 14.4 and 3.26%, respectively. The use of the P_4 and E_2 kits for chicken has previously validated by Onagbesan et al. (2006). Plasma testosterone was determined using a RIA kit (DRG, Marburg/Lahn, Germany) according to manufacturer procedure with an intraassay CV of 13.05%. Total T_3 and T_4 concentrations were determined using commercial RIA kits (DRG). The intraassay CV for T_3 and T_4 were 4.90 and 7.67%, respectively. Plasma leptin-like concentration and glucagon were determined at the ages of 27 (before initiation of experiment), 33, 37, and 39 wk, whereas all of the other metabolites and hormones were measured every 2 wk from 27 to 39 wk of age.

Statistical Analysis

The experimental design was a completely randomized design using individual broiler breeder hens as experimental units. There were 4 methods of feed allocation and 7 separate periods of observation. Performance data as well as plasma metabolite and hormone data were analyzed as repeated measures using PROC MIXED of SAS software (SAS Institute, 2002). Differences between means were evaluated using least squares means procedure. Feed intake data and data obtained in necropsy were analyzed using the GLM procedure of SAS. Means were separated by Duncan's multiple range test. Significant differences were considered to be $P < 0.05$.

RESULTS

Feed Intake and BW

Mean feed intake every 14 d and BW at 39 wk of age are presented in Table 1. Hens with free access to the feed consumed significantly ($P < 0.05$) more feed than their restricted counterparts. They also weighed significantly more compared with restricted-fed hens. Broiler breeder hens received their restricted feed 2 and 3 times per day and had significantly ($P < 0.05$) lower BW than the R1D group from 35 wk and thereafter (except for R2D at 37 wk of age; data not shown).

Organ Weight and Ovarian Follicles

Feed-satiated hens had significantly ($P < 0.05$) higher abdominal fat pad weights than R1D, R2D, and R3D hens ($P < 0.05$). Interestingly, the R2D broiler breeder hens stored less ($P < 0.05$) fat in the abdominal cavity compared with the R1D hens (Table1). Liver fractional weight in the ALF broiler breeders was significantly higher ($P < 0.05$) than their restricted-fed counterparts; the liver relative weight of R2D hens was significantly lower than R1D hens (Table 1). Feed-satiated hens had significantly more LYF and less small yellow follicles and LWF in comparison to the feed-restricted hens ($P < 0.05$). The R2D hens had more ($P < 0.05$) LWF than the R1D birds (Table 2).

Egg Production, Egg Weight, and Yolk Weight

For the entire production period, the overall percentage of hen-day egg production was significantly greater ($P < 0.05$) for the R2D hens compared with the hens from the R1D, R3D, and ALF feeding regimens (Figure 1A). Mean cumulative egg production of R2D hens was 6.76 ($P < 0.04$), 19.26 ($P < 0.0001$), and 27.67 ($P < 0.0001$) more than R1D, R3D, and ALF hens, respectively. The R3D hens failed to maintain egg production through the production period and a significant depression in their egg production occurred at 33 wk of age, which continued until experiment termination. However, none of the hens from the R3D regimen ceased to lay until termination of the experimental period. They produced significantly lower eggs than R1D ($P = 0.0003$) and R2D ($P < 0.0001$) hens in the entire production period (Figure 1B). Although hens with free access to feed produced even numerically more eggs early in the production period (until 29 wk of age) compared with restricted-fed birds, their infe-

Table 1. Carcass and performance data[1]

Treatment[2]	Mean feed intake/2 wk (kg)	BW at 39 wk of age (kg)	Fat pad fractional weight[3] (%)	Liver fractional weight[3] (%)	Double yolk egg (%)	Soft-shell egg (%)
ALF	2.38 ± 0.09[a]	4.06 ± 0.05[a]	3.19 ± 0.30[a]	2.04 ± 0.09[a]	1.65 ± 0.60[a]	2.80 ± 0.80[a]
R1D	1.80 ± 0.06[b]	3.58 ± 0.01[b]	2.36 ± 0.15[b]	1.68 ± 0.03[b]	0.38 ± 0.21[b]	0.38 ± 0.21[b]
R2D	1.80 ± 0.05[b]	3.43 ± 0.06[c]	1.41 ± 0.20[c]	1.33 ± 0.10[c]	0.12 ± 0.12[b]	0.38 ± 0.21[b]
R3D	1.80 ± 0.06[b]	3.36 ± 0.06[c]	1.73 ± 0.13[bc]	1.48 ± 0.12[bc]	0.38 ± 0.21[b]	0.63 ± 0.27[b]

[a–c]Means within columns with different superscripts are significantly different ($P < 0.05$).

[1]All values represent the mean ± SEM.

[2]ALF = ad libitum feeding; R1D = restricted feed once a day; R2D = restricted feed twice a day; R3D = restricted feed 3 times a day.

[3]Fat pad and liver fractional weights expressed as percentage of BW.

rior egg production thereafter resulted in significantly ($P < 0.01$) lower egg production in the entire production period (Figure 1). Ad libitum feeding increased egg weight, yolk weight, yolk fractional weight (except for R1D; Table 2), and numbers of double-yolk and soft-shell eggs (Table 1) compared with restricted-fed birds ($P < 0.05$). The R2D birds produced heavier eggs than the R1D and R3D ($P < 0.05$) hens. However, fractional yolk weight was not different among the R1D, R2D, and R3D birds (Table 2).

Plasma Glucose, TAG, and Cholesterol Concentration

Plasma glucose concentration of samples obtained at 1030 and 1330 h bleeding were not significantly different and therefore were pooled. As feeding frequency increased to 2 or 3 times a day, plasma glucose concentration (Figure 2) decreased to the level even lower (numerically) than the level at 27 wk of age (before allocation of feeding regimen). The R2D as well as R3D hens had significantly lower glucose than R1D hens from 31 wk of age and thereafter ($P < 0.0001$), except for R2D at 35 wk of age. Plasma glucose concentration of feed-satiated hens increased dramatically as feed was provided ad libitum and remained at a high level through 39 wk of age ($P < 0.0001$; Figure 2). Hens from the ALF group had significantly higher plasma triglyceride (**TG**, $P < 0.0001$; Figure 3) and cholesterol ($P < 0.0001$; Figure 4) concentration than their restricted-fed counterparts. Although R3D hens had significantly lower TG than R1D hens at 37 and 39 wk of age, feeding frequency did not affect plasma TG concentration at the other ages. However, when plasma TG concentration was compared in an entire production period, increasing feeding frequency (R2D and R3D) resulted in significantly ($P < 0.0001$) lower plasma TG level compared with feeding once a day (Figure 3). Plasma cholesterol concentrations were not significantly different between hens with restricted feeding 1, 2, or 3 times a day.

Hormone Levels

The hens from the ALF treatment had a significantly higher plasma leptin-like concentration at 33 ($P < 0.0001$), 37 ($P < 0.02$), and 39 ($P < 0.05$) wk of age than their restricted-fed counterparts (Table 3). Hens fed 2 or 3 times a day had a significantly ($P < 0.0001$) lower plasma leptin-like concentration than those that received their feed once a day (Table 3; except for R2D at 33 wk of age).

Glucagon concentrations of hens fed on different feeding regimens are shown in Table 3. Ad libitum-fed broiler breeder hens had significantly ($P < 0.0001$) lower plasma glucagon concentrations at 33, 37, and 39 wk of age compared with hens fed on R1D, R2D, and R3D feeding programs. The plasma glucagon concentration

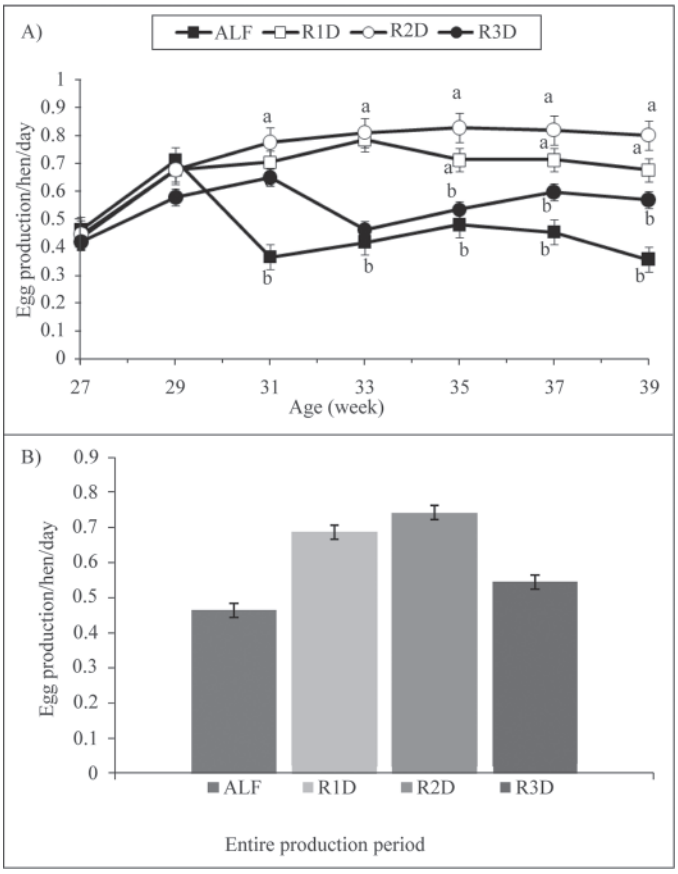

Figure 1. Mean actual egg production/hen per day in every 2-wk period (panel A) and entire production period (panel B) of hens that were fed ad libitum (ALF) and restricted feed once a day (R1D), twice a day (R2D), and 3 times a day (R3D). Data are means ± SEM. [a,b]Data points with different letters are significantly different at the age of the hens ($P < 0.05$).

Table 2. Egg and ovarian parameters[1]

Treatment[2]	Mean egg weight (g)	Mean yolk weight (g)	Fractional yolk weight (%)	Large yellow follicles per ovary	Small yellow follicles per ovary	Large white follicles per ovary
ALF	62.64 ± 0.72[a]	17.89 ± 0.27[a]	28.82 ± 0.43[a]	9.50 ± 0.50[a]	10.0 ± 1.0[b]	9.5 ± 1.8[c]
R1D	58.85 ± 0.62[c]	16.55 ± 0.23[b]	28.02 ± 0.37[ab]	6.50 ± 0.50[bc]	14.50 ± 0.5[a]	19.6 ± 0.5[b]
R2D	60.64 ± 0.64[b]	16.29 ± 0.24[b]	27.12 ± 0.39[b]	7.33 ± 0.33[b]	14.66 ± 0.8[a]	28.5 ± 2.3[a]
R3D	58.53 ± 0.63[c]	16.08 ± 0.23[b]	27.60 ± 0.38[b]	5.33 ± 0.33[c]	13.66 ± 0.8[a]	25.0 ± 2.8[ab]

[a–c]Means within columns with different superscripts are significantly different ($P < 0.05$).

[1]All values represent the mean ± SEM.

[2]ALF = ad libitum feeding; R1D = restricted feed once a day; R2D = restricted feed twice a day; R3D = restricted feed 3 times a day.

of R2D hens was significantly higher ($P < 0.01$) than R1D hens at 37 and 39 wk of age.

Plasma E_2 concentration in R2D hens (Figure 5) tended to be higher in the later stage of the experiment compared with hens from the R1D regimen (differences at 37 wk were significant and at 39 wk were numerical); however, entire production period analysis revealed that R2D hens had significantly a higher E_2 concentration than R1D and R3D hens (Figure 5). Free access to feed resulted in a significantly lower E_2 concentration in ALF hens compared with restricted-fed birds ($P < 0.0001$). Contrary to E_2 concentration, plasma testosterone concentrations (Figure 6) during the postpeak period (37 and 39 wk of age) in R2D hens were significantly lower than R1D hens ($P < 0.04$). Ad libitum feeding resulted in a significantly higher testosterone concentration in ALF hens ($P < 0.0001$).

Feeding regimen had no significant effect on plasma basal P_4 concentration (Figure 7); however, the effect of week on P_4 concentration ($P < 0.0001$) and the interaction between week and feeding regimen ($P < 0.0001$) were significant. Plasma P_4 peak concentration in ALF broiler breeder hens occurred 2 wk earlier than P_4 peak concentration in restricted-fed hens.

Neither ad libitum feeding nor feeding frequency (R1D, R2D, and R3D) had a significant effect on plasma T_3 and T_4 concentration (data not shown).

DISCUSSION

Performance Data

Similar to previous reports (Chen et al., 2006; Sun et al., 2006), release from feed restriction in our study resulted in hens that were approximately 500 g heavier at 39 wk of age compared with their restricted-fed counterparts. Increasing feed allocation to 2 or 3 times a day also resulted in significantly lower BW compared with the R1D feeding regimen. Spradley et al. (2008) demonstrated that increasing feeding frequency to 2 times a day decreased BW through 42 wk of age, whereas the same pattern did not continue thereafter.

Hens with free access to feed had comparable egg production with feed-restricted birds early in the production period (during 27 to 29 wk), but their egg production decreased dramatically thereafter. Decreased egg production in broiler breeders with ad libitum feeding is well documented (Yu et al., 1992; Robinson et al., 1993; Chen et al., 2006; Onagbesan et al., 2006; Sun et al., 2006).

Although no significant differences in egg production were observed between R1D and R2D hens during every 2-wk period, the R2D hens produced significantly more eggs in the entire production period. In the same period, Spradley et al. (2008) came to similar results.

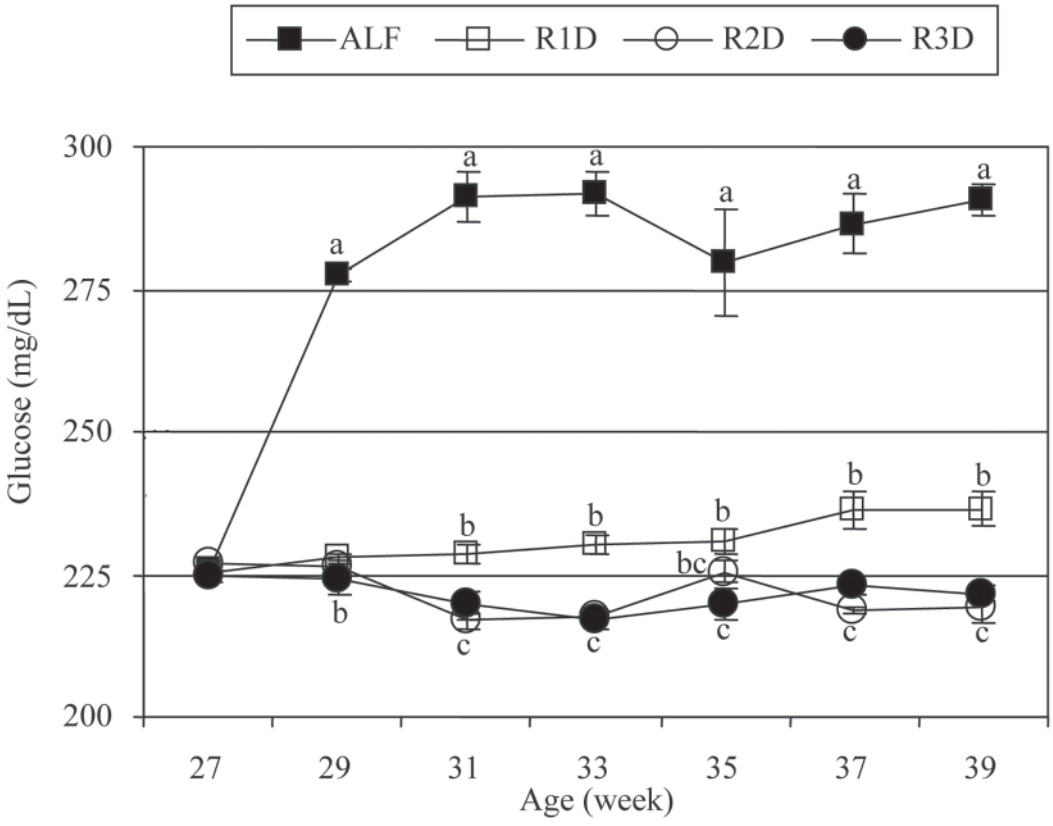

Figure 2. Plasma glucose concentrations (mg/dL) of hens that were fed ad libitum (ALF) and restricted feed once a day (R1D), twice a day (R2D), and 3 times a day (R3D). Data are means ± SEM (n = 16). [a–c]Data points with different letters are significantly different at the age of the hens ($P < 0.05$).

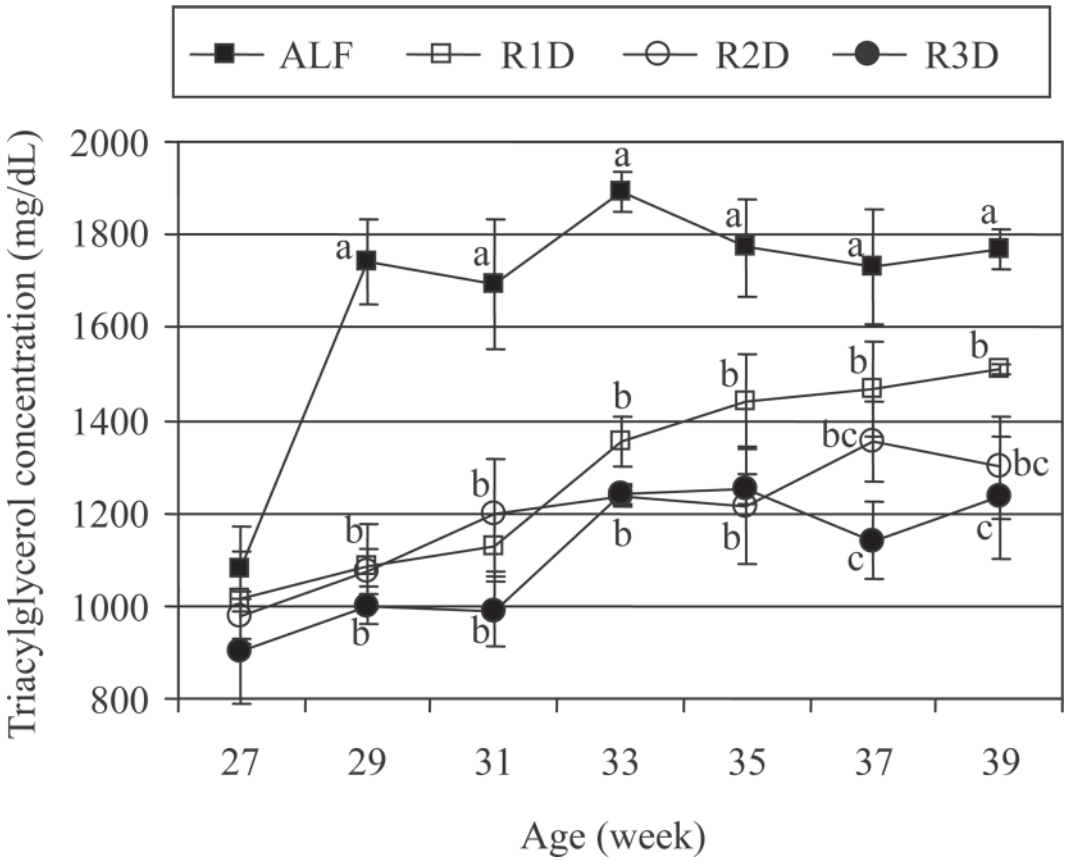

Figure 3. Plasma triacylglycerol concentrations (mg/dL) of hens that were fed ad libitum (ALF) and restricted feed once a day (R1D), twice a day (R2D), and 3 times a day (R3D). Data are means ± SEM (n = 8). [a–c]Data points with different letters are significantly different at the age of the hens ($P < 0.05$).

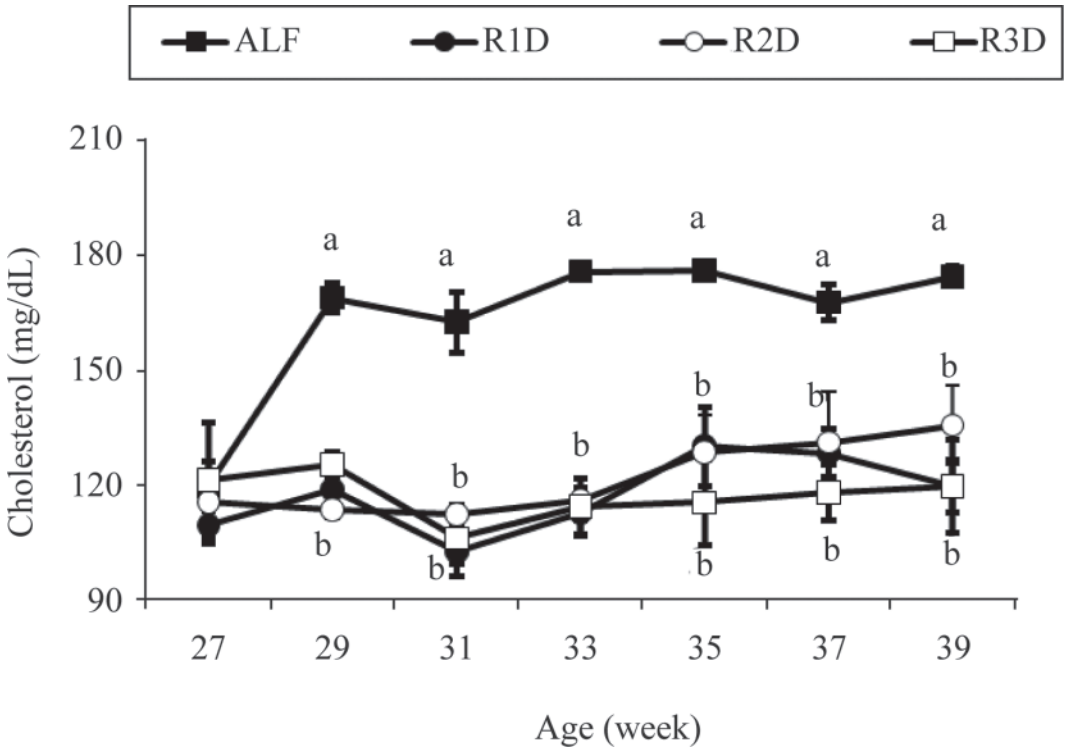

Figure 4. Plasma cholesterol concentrations (mg/dL) of hens that were fed ad libitum (ALF) and restricted feed once a day (R1D), twice a day (R2D), and 3 times a day (R3D). Data are means ± SEM (n = 8). [a,b]Data points with different letters are significantly different at the age of the hens ($P < 0.05$).

Cave (1981) reported that feeding broiler breeder hens 3 times a day increased the percentage of hen-day egg production for the first 10 wk of production when compared with hens fed once or twice a day. As previously reported by Morris and Nalbandov (1961), better performance in twice-a-day-fed birds may be partly due to a shortened fasting period.

The percentage of abnormal eggs including double-yolk and soft-shell eggs was higher in unrestricted-fed birds. Feed-satiated hens produced heavier eggs with higher yolk absolute and fractional weights. As demonstrated by Chen et al. (2006), an observed increase in actual and fractional yolk weight coupled with decreased egg production may be a consequence of prolonged retention of follicles within the hierarchy of feed-satiated hens.

The observed increase in egg weight for hens fed twice a day compared with those fed once a day in our study is consistent with the reports by Cave (1981) and Spradley et al. (2008). Although Spradley et al. (2008) believed that increased egg weight was related to providing feed later in the day (1500 h) rather than feeding the hens twice a day, our results suggest that increased egg weight may be partly due to more efficient utilization of feed due to increased feeding frequency because the second meal in our study was allocated at 1130 h. Increased feeding frequency has been shown to improve feed utilization in pigs through improvements in nutrient digestibility (Fanimo et al., 2003). Because of similar yolk absolute and fractional weight in eggs from R1D and R2D hens, increased egg size should be related to an increase in egg components other than yolk. The inability of the R3D feeding program to increase egg weight may be explained by the reason mentioned previously, that nutrient deficiency occurred in these hens.

Necropsy revealed that feed-satiated hens had higher fractional fat pad and liver weights (Table 1). They also had significantly more LYF and lower LWF compared with feed-restricted birds. These results were consistent with previous reports (Yu et al., 1992; Chen et al., 2006; Sun et al., 2006). The dramatically increased liver and fat pad fractional weights are likely due to excessive lipid synthesis and accumulation (because of high glucose availability) in the liver and abdominal cavity of feed-satiated hens.

The R2D hens stored less fat in the abdominal cavity and had significantly lower liver fractional weight compared with the R1D birds. These results are in agreement with lower plasma glucose, leptin-like concentration, and TG in R2D compared with R1D hens. Hyperglycemia, hyperleptinemia, and hyperlipidemia are known to be positively correlated to adiposity in mammals (Ahima and Flier, 2000).

Hormones and Metabolites

Results obtained in the current study and also results from addition of metformin (a glucose-lowering agent)

Table 3. Plasma leptin and glucagon concentrations[1]

	Leptin (ng/mL)				Glucagon (ng/mL)			
Treatment[2]	27 wk	33 wk	37 wk	39 wk	27 wk	33 wk	37 wk	39 wk
ALF	8.90 ± 0.74	13.40 ± 0.74^{a}	12.54 ± 0.74^{a}	12.75 ± 0.74^{a}	6.94 ± 0.27	4.69 ± 0.57^{c}	4.80 ± 0.64^{c}	5.67 ± 0.46^{c}
R1D	8.80 ± 0.74	10.40 ± 0.74^{b}	10.80 ± 0.74^{b}	11.30 ± 0.74^{b}	7.59 ± 0.33	7.31 ± 0.34^{b}	7.33 ± 0.38^{b}	7.13 ± 0.26^{b}
R2D	10.02 ± 0.74	9.00 ± 0.74^{bc}	8.20 ± 0.74^{c}	8.20 ± 0.74^{c}	7.45 ± 0.30	7.83 ± 0.62^{ab}	8.77 ± 0.44^{a}	8.47 ± 0.48^{a}
R3D	9.00 ± 0.74	8.70 ± 0.74^{c}	8.70 ± 0.74^{c}	8.60 ± 0.74^{c}	7.38 ± 0.33	8.68 ± 0.42^{a}	8.04 ± 0.73^{ab}	7.84 ± 0.42^{ab}

$^{a-c}$Means within columns with different superscripts are significantly different ($P < 0.05$).

[1]All values represent the mean ± SEM (n = 8).

[2]ALF = ad libitum feeding; R1D = restricted feed once a day; R2D = restricted feed twice a day; R3D = restricted feed 3 times a day.

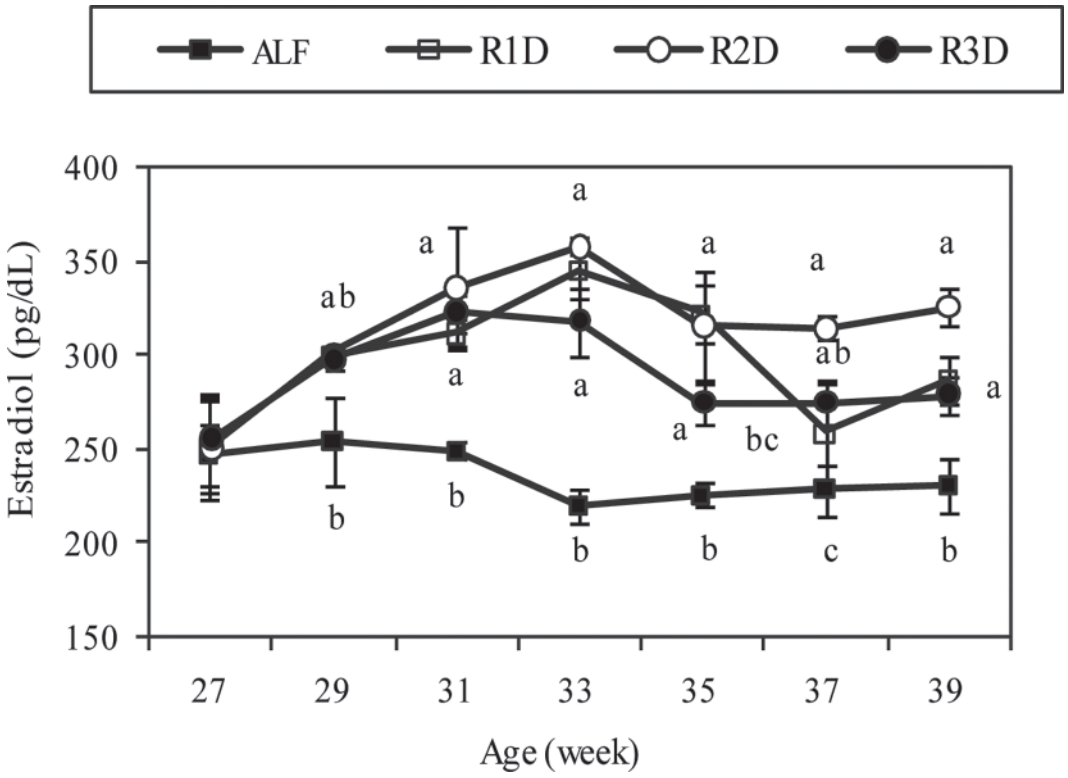

Figure 5. Plasma estradiol concentrations (pg/dL) of hens that were fed ad libitum (ALF) and restricted feed once a day (R1D), twice a day (R2D), and 3 times a day (R3D). Data are means ± SEM (n = 8). [a–c]Data points with different letters are significantly different at the age of the hens ($P < 0.05$).

to broiler breeder diets (R. Taherkhani, unpublished data) suggest that plasma glucose concentration may be involved in the regulation of reproductive performance. Better egg production in the R2D compared with the R1D group was associated with decreased plasma glucose, leptin-like concentration, and TG (in the entire

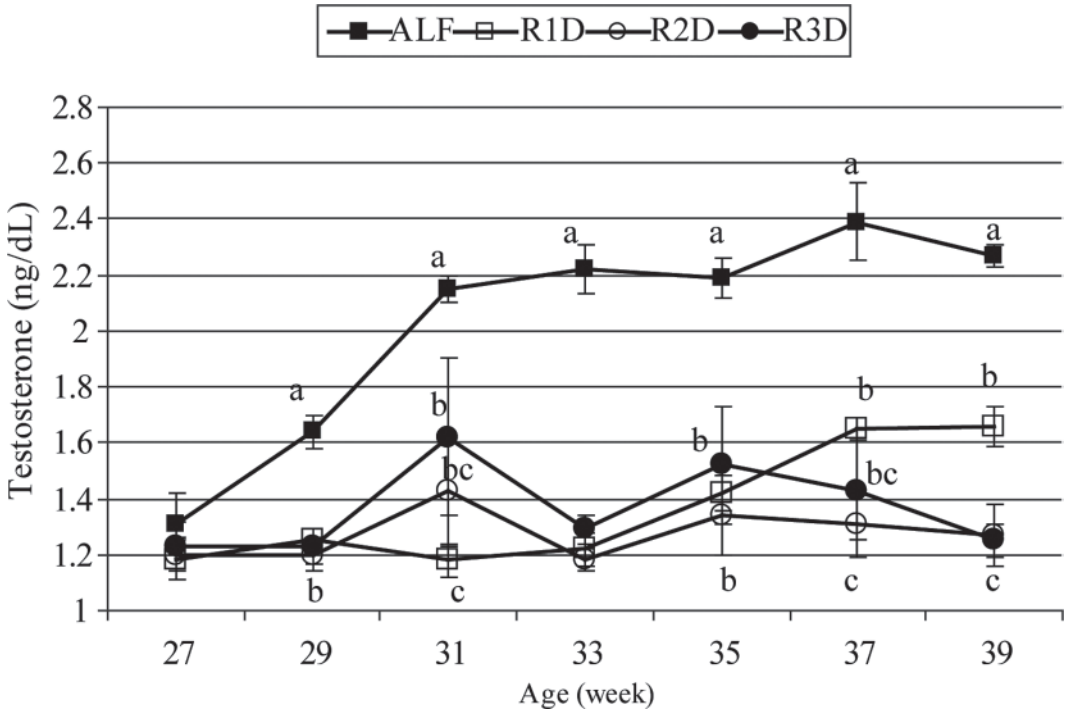

Figure 6. Plasma testosterone concentrations (ng/dL) of hens that were fed ad libitum (ALF) and restricted feed once a day (R1D), twice a day (R2D), and 3 times a day (R3D). Data are means ± SEM (n = 8). [a–c]Data points with different letters are significantly different at the age of the hens ($P < 0.05$).

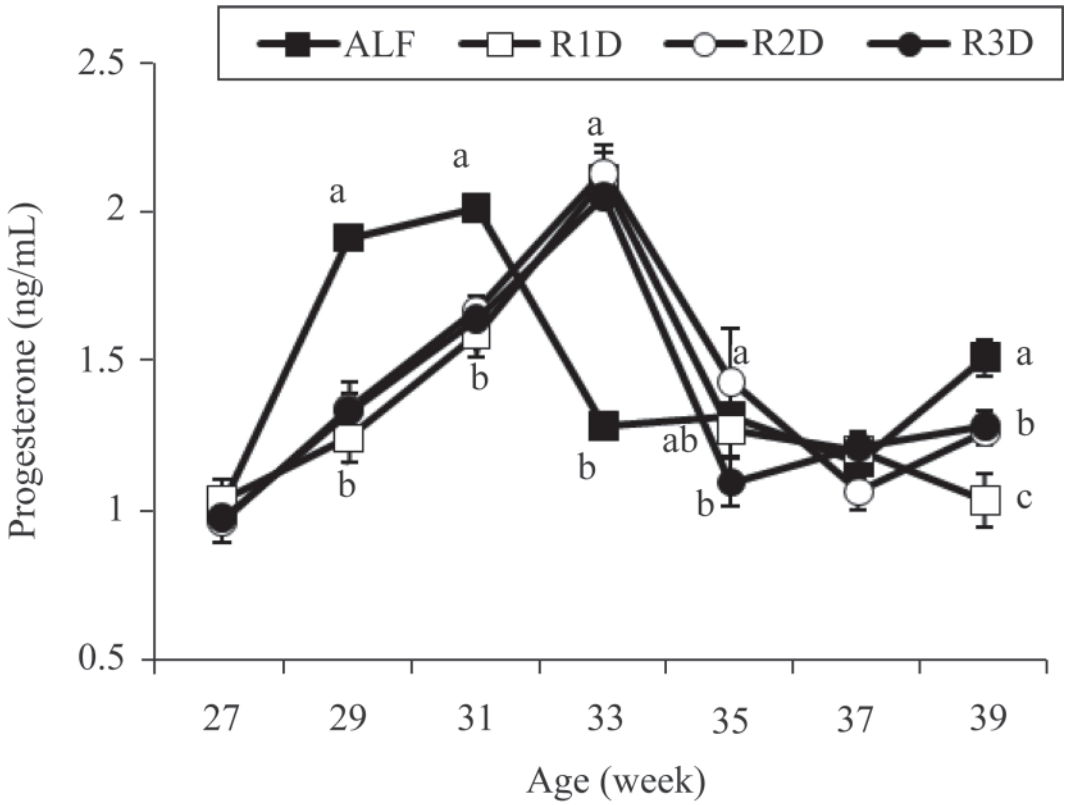

Figure 7. Plasma progesterone concentrations (ng/mL) of hens that were fed ad libitum (ALF) and restricted feed once a day (R1D), twice a day (R2D), and 3 times a day (R3D). Data are means ± SEM (n = 8). [a–c]Data points with different letters are significantly different at the age of the hens ($P < 0.05$).

production period; Figure 3). The R3D feeding regimen also decreased plasma glucose, TG, and leptin-like concentration. In patients with type 2 diabetes, Wadhwa et al. (1973) reported that the more frequent eating pattern, in which a more constant energy supply is provided by the diet, resulted in lower blood glucose, TG, and cholesterol levels. Ad libitum-fed birds had free access to feed before both blood samplings. Hens from the R1D, R2D, and R3D groups received 100, 50, and 33.33%, respectively, of their total daily feed intake before first blood sampling. Before second blood sampling, hens from R1D received 0, 50, and 33.33% of their total daily feed intake. Feed cleanup time for R1D, R2D, and R3D hens was 65, 35, and 38 min, respectively. It seems that decreased amount of feed in each meal in R2D and R3D hens compared with R1D birds is likely an important factor affecting blood glucose, TG, and cholesterol concentrations. To our knowledge, there is no published data on the effects of increased feeding frequency on plasma metabolite and hormone levels in broiler breeder hens.

Decreased egg production in ALF hens was associated with higher plasma glucose, TG, and leptin-like concentration. Chen et al. (2006) demonstrated that high glucose availability in feed-satiated broiler breeders increased hepatic de novo lipogenesis and triggered lipotoxicity development and ovarian dysfunction. Lipotoxicity is associated with reduced insulin sensitivity (type 2 diabetes), hyperglycemia, hyperlipedimia, hyperleptinemia, and hyperinsulenemia. Reports by Ashwell et al. (1999), Taouis et al. (2001), and Sun et al. (2006) also agree with the data reported herein (Table 3) that feed-satiated broiler breeders had a higher plasma leptin-like level.

Feeding frequency also significantly affected plasma leptin-like level. Plasma leptin-like concentration decreased significantly as feeding frequency increased to 2 or 3 times a day. Ashwell et al. (2001) reported that metabolic BW and adipose leptin expression were positively correlated. Furthermore, the concentrations of leptin in plasma have been shown to be a direct reflection of the amount of body fat (Dagogo-Jack et al., 1996; McGregor et al., 1996). In broiler breeder pullets, de Beer et al. (2008) have shown that providing a more consistent food supply and more stable energy status through an everyday feeding program in comparison with skip-a-day feed allocation resulted in lower leptin-like concentration.

Lower BW and fat pad weight in R2D and R3D hens concurred with the plasma leptin-like levels. The same argument is true about leptin-like concentration, BW, and abdominal fat pad in ALF and R1D birds.

Leptin appears to inhibit ovarian function on the basis of a variety of parameters. For example, it inhibits the insulin-induced secretion of P_4 and androstenedione from cultured bovine theca cells (Spicer and Francisco, 1998) and the insulin-induced production of E_2 and P_4 from bovine granulosa cells (Spicer and Francisco,

1997). Zachow and Magoffin (1997) showed an inhibitory effect of leptin on combined insulin-like growth factor-I- and follicle-stimulating hormone-stimulated estrogen production from rat granulosa cells. In cultured human granulosa cells, Karlsson et al. (1997) found that leptin inhibited luteinizing hormone-stimulated E_2 secretion. In poultry, Paczoska-Eliasiewicz et al. (2003) showed that prolonged leptin treatment during the refeeding period significantly hampered follicle entry into the hierarchy and follicle growth delaying ovarian restoration.

Although feeding regimen had no significant effect on plasma basal P_4 concentration, feed allocation method significantly affected plasma E_2 and testosterone levels. The interesting observation was higher plasma testosterone concentrations in ALF hens, whereas their E_2 concentration was lower than their restricted-fed counterparts. As egg production began to decrease in R1D hens, their plasma testosterone concentration tended to increase and simultaneously E_2 concentration tended to decrease. However, differences between R1D and R2D were significant only at 37 and 39 wk for testosterone and 39 wk and also the entire production period (Figure 5) for E_2 level.

The lower E_2 concentration of ALF hens compared with restricted-fed hens observed in this research is in agreement with Onagbesan et al. (2006). Lower E_2 level in ALF and R1D hens compared with R2D hens are consistent with their LWF number (LWF are the main secretor of E_2).

Higher testosterone levels in ALF and R1D hens compared with R2D and R3D hens suggest that a lipotoxicity-dependent factor may impair conversion of androgen to E_2. As previously discussed, leptin may be a good candidate to impair conversion of androgen to E_2 because negative effect of high leptin concentration on E_2 production is well documented (Karlsson et al., 1997; Spicer and Francisco, 1997; Zachow and Magoffin, 1997).

Higher glucose level was associated with lower glucagon level in ALF and R1D hens compared with R2D and R3D hens. Higher plasma glucagon in feed-restricted birds compared with ALF birds observed in our study agrees with reports by Chen et al. (2006) and Sun et al. (2006). It has been shown that glucagon inhibits hepatic lipogenesis (Rosebrough and McMurtry, 1992) and stimulates lipolysis in adipose tissue (McMurtry et al., 1996). Lower glucagon concentration in ALF birds in comparison to restricted-fed hens was associated with higher abdominal fat pad weight.

Numerous studies suggest that feed restriction decreases T_3 and increases T_4 levels in broiler breeders (Darras et al., 1995; Bruggeman et al., 1997). Bruggeman et al. (1997) showed that T_3 concentrations decreased and T_4 concentrations increased in both restricted- and ad libitum-fed chicks during ontogeny (2 to 24 wk). However, consistent with our results, Sun et al. (2006) did not observe a significant difference in T_3 and T_4 concentration of broiler breeder hens fed either ad libitum or restricted from first egg to 36 wk of age. They concluded that effect of feeding regimen on plasma T_3 and T_4 is age-related.

In conclusion, allocation of restricted feed 2 times a day improved glucose homeostasis and decreased plasma glucose level. Lower glucose availability is associated with decreased de novo lipogenesis and leaner body mass. Obesity along with high leptin level may impair ovary steroid biosynthesis, gonadotropin secretion, and also follicle rupture and ovulation. Our results suggest that increased feeding frequency could improve reproductive performance of broiler breeder hens through preventing or delaying lipotoxicity development.

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