Effects of Body Weight and Feed Allocation During Sexual Maturation in Broiler Breeder Hens. 2. Ovarian Morphology and Plasma Hormone Profiles

R. A. RENEMA,* F. E. ROBINSON,*,[1] J. A. PROUDMAN,† M. NEWCOMBE,‡ and R. I. McKAY‡

*Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, T5G 2P5;
†Germplasm/Gamete Physiology Laboratory, USDA/ARS, Beltsville, Maryland, 20705, and
‡Shaver Poultry Breeding Farms Ltd., Cambridge, Ontario, Canada, N1R 5V9

ABSTRACT The effects of broiler breeder BW and nutrient intake on ovary morphology and plasma reproductive hormone profiles were examined at photostimulation (PS) (21 wk) and at sexual maturity (SM) in standard (STD) and low (LOW), or high (HIGH) BW birds provided either restricted (RF) or *ad libitum* (AL) access to feed between PS and SM. At PS, 30 Shaver Starbro pullets at target BW were assigned to the STD treatment, and birds either 20% heavier (HIGH) or lighter (LOW) assigned accordingly. Ten birds of each size group were processed immediately for carcass analysis and 10 birds assigned to each size by feed interaction group. Blood samples were taken at 3-d intervals beginning at PS and profiles constructed for estradiol-17β, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) to examine the relationship between body size, feeding level, and reproduction. Birds were processed for assessment of reproductive traits following SM.

The AL birds reached SM with 11.0 large yellow follicles (LYF) (> 10 mm diameter) compared to 7.1 in RF birds. Small follicle atresia (< 5 mm diameter) was low in AL birds (10.3) compared to RF birds (32.3). The extent of small follicle atresia in RF birds was found to be inversely proportional to LYF number by stepwise regression. Increased small follicle atresia was associated with a longer sexual maturation period in RF birds (r = 0.619; P = 0.0003). Plasma estradiol-17β concentration was greater in HIGH than in STD or LOW birds at PS, suggesting more advanced ovary development in HIGH birds. Estradiol-17β profiles were similar in shape in all treatments, with the primary difference being the length of time prior to a substantial estradiol-17β increase. Following PS, plasma LH and FSH concentrations of AL birds increased to levels nearly double that of RF birds, indicating a role for nutrient intake with rate of reproductive development. Plasma LH and FSH concentrations remained elevated for a greater time period in RF birds, however, possibly relating to the development of processes limiting LYF recruitment. This experiment demonstrated a modulation of reproductive hormone concentrations during sexual maturation by feeding level in conjunction with a sensitivity of the ovary to nutritional effects.

(*Key words*: broiler breeder, ovary morphology, estradiol-17β, luteinizing hormone, follicle-stimulating hormone)

INTRODUCTION

Broiler breeder BW is routinely controlled from an early age to reduce reproductive problems associated with genetic selection for growth. Overfeeding growth-selected poultry during reproductive development results in the formation of excess large yellow ovarian follicles (LYF), which are more likely to be arranged in multiple hierarchies of large follicles (Hocking *et al.*, 1987; Katanbaf *et al.*, 1989; Yu *et al.*, 1992a) and result in increased production of unsettable eggs. The sensitivity of the ovary to overfeeding is most prevalent near

sexual maturity (SM). By 44 wk of age, excess follicular recruitment occurs to a lesser degree than at SM (Robinson *et al.*, 1993), and by 54 wk of age, ovarian morphology appears to be insensitive to moderate overfeeding (McGovern *et al.*, 1997).

During reproductive development, the number of LYF may be sensitive to timing of SM, feeding level, and BW. Early maturing birds have been found to have increased LYF number (Hocking *et al.*, 1988) and more multiple LYF hierarchies (Hocking, 1992; Renema *et al.*,

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Abbreviation Key: AL = *ad libitum* feeding; D = dark; FSH = follicle-stimulating hormone; HIGH = high BW; L = light; LH = luteinizing hormone; LOW = low BW; LWF = large white follicles; LYF = large yellow follicles; MWF = medium white follicles; POF = post-ovulatory follicles; PS = photostimulation; RF = restricted feeding; SM = sexual maturity; STD = standard BW; SYF = small yellow follicles.

1998) compared to later maturing birds. However, the primary effector of LYF number appears to be BW (Hocking and Whitehead, 1990; Hocking, 1993, 1996). A higher feeding level will elevate LYF number in birds of similar BW (Hocking, 1993), and will accelerate the sexual maturation process (Wilson and Harms, 1986; Yu *et al.*, 1992a). Low BW birds in a flock have been found to commence lay later and lay fewer eggs than medium- or high-weight hens (Robinson and Robinson, 1991). Monitoring changes in ovarian morphology parameters between photostimulation (PS) and SM in birds varying in BW may yield specific information on the reproductive disadvantage of birds with a small BW at PS. In this experiment, the effects of broiler breeder BW on ovarian morphology and plasma reproductive hormone profiles were examined at PS and SM in standard and naturally low or high BW birds. Hormone profiles were constructed for estradiol-17β, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) to examine the effects of feed and body size on the profiles and their potential relationship with ovarian morphology and carcass composition at SM. The interaction of *ad libitum* feeding with variation in BW was also examined to compare the influence of excess nutrient availability with restricted feeding conditions and their effects on the relationship between nutrition and reproduction.

MATERIALS AND METHODS

Stocks and Management

A flock of 420 Shaver Starbro[2] broiler breeder pullets was reared in six 4.75×5.85 m floor pens in a light-tight facility. Birds were allowed *ad libitum* access to feed for 2 wk, after which they were feed restricted using skip-a-day feeding to align BW gains with breeder recommended targets. A starter diet was fed until 3 wk of age (2,875 kcal ME, 18% CP), followed by a grower diet until 21 wk of age (2,700 kcal ME, 15% CP), and a breeder diet from 21 wk of age until processing (2,750 kcal ME, 16% CP). Body weight was monitored by recording individual BW at 4-wk intervals and group weights were taken all other weeks. Pullets received 24 h of light (L):0 h dark (D) for the first 24 h, which was decreased to 8L:16D until PS.

A detailed description of the experimental design has been presented elsewhere (Renema, *et al.*, 1999). Briefly, birds were individually weighed at 20 wk of age and the data sorted by BW. Thirty birds near the pen mean were selected for the standard (STD), target BW group. An additional 30 birds were selected from the top and bottom of the BW distribution to represent birds 20% lighter (LOW) or 20% heavier (HIGH) than STD birds. Ten birds were randomly selected from each size group at PS (21 wk of age) and processed to assess the degree of reproductive

development. Birds were euthanatized by cervical dislocation, and the oviduct and ovary dissected and weighed.

The remaining 20 birds within each size group were randomly assigned to either a standard restricted feeding regimen (RF), or an *ad libitum* feeding program (AL), resulting in a 2×3 factorial experimental design with feed allocation (AL and RF) and body size (LOW, STD, and HIGH) as the main effects. Birds were randomly assigned to individual cages and photostimulated. The feed of the RF-STD birds was allocated to maintain birds on the breeder-recommended target BW curve and feed for the LOW and HIGH RF birds was allocated to maintain similar rates of BW gain to the STD birds. Feed increases of 4 g or more were divided up into two to three smaller increases per week. Individual feed intake and BW was monitored for all birds. The experimental protocol was approved by the Animal Policy and Welfare Committee of the Faculty of Agriculture, Forestry, and Home Economics of the University of Alberta.

Carcass Traits at Sexual Maturity

Birds were maintained on assigned feeding regimens until first oviposition, following which each bird was euthanatized. The oviduct and ovary were removed and weighed. The number and weight of normal LYF (> 10 mm diameter), and the number of small yellow follicles (SYF) (5 to 10 mm diameter), large white follicles (LWF) (3 to 5 mm diameter), and medium white follicles (MWF) (1 to 3 mm diameter) were recorded. Follicle size classifications were based on previous reports (Robinson and Etches, 1986), although in the current study the LWF range was continuous with the SYF, and MWF denoted follicles 1 to 3 mm in diameter. Stroma weight was recorded with the LYF removed (initial stroma) and again when the SYF, LWF, and MWF had been removed (bare stroma) to assess treatment effects on large and small follicle types separately.

An assessment of the potential for multiple ovulations to occur was determined by assigning LYF of similar size (differing by less than 1 g or 1 mm diameter) to the same position in the hierarchy as reported previously (Renema *et al.*, 1995). Total number of positions and proportion of follicles in a multiple hierarchical arrangement were recorded. Number of complete hierarchies of LYF was calculated by dividing LYF number by the number of positions in the hierarchy. The number of postovulatory follicles (POF) on the stroma was recorded. Unexplained ovulations, defined as ovulations occurring prior to first oviposition, were calculated by subtracting any eggs previously laid or in the oviduct from the number of POF found at processing. The incidence of internal ovulation (as evidenced by yolk residue in the body cavity) was assessed. Follicles with a discolored or shrunken appearance were considered atretic (Gilbert *et al.*, 1983). The incidence of follicular atresia of the yellow follicles (> 5 mm diameter) and of the small follicles (< 5 mm diameter) was assessed by counting all visibly atretic follicles in the appropriate ranges.

Plasma Lipid and Hormone Analysis

Blood samples were taken from each bird at PS and SM using EDTA-coated vacuum blood collection tubes. Blood was centrifuged at $1,500 \times g$ for 20 min at 3 C and stored at –30 C until plasma lipids were quantified. Total plasma lipid weight was determined by Folch extraction. Chloroform was evaporated from the 15-mL extracted aliquot under a nitrogen stream on a hotplate (70 C at surface) until a constant weight was achieved.

Beginning at PS, all birds were blood sampled at 3-d intervals to monitor changes in plasma estradiol-17β, LH, and FSH concentrations. Plasma estradiol-17β concentration was determined by RIA[3] using duplicate 200 μL samples in four assays. Assay parallelism was determined by measuring estradiol-17β concentration in various plasma volumes. For 50, 100, 200, and 400 μL of plasma, estradiol-17β concentration (mean $\pm$ SEM) was 112 ± 5, 184 ± 3, 328 ± 9, and 647 ± 4 pg. Sample duplicate variation of up to 5% was allowed. The interassay coefficient of variation was 5.89% and the intra-assay coefficient of variation was 3.69%. The assay sensitivity was 1.5 pg/mL. The antiserum was highly specific for estradiol-17β with a relatively low cross reactivity to other naturally occurring steroids in the plasma sample as stated by the manufacturer. All tested compounds had a cross-reactivity of less than 1% with the exception of d-Equilenin (4.4%), Estrone (10%), Estrone-β-D-glucuronide (1.8%), and Ethinyl estradiol (1.8%). The LH and FSH determinations were performed using the methods of Krishnan *et al.* (1994) and Krishnan *et al.* (1993), respectively. The inter- and intra-assay coefficients of variation for the LH assays were 10.51 and 10.47%, respectively, whereas for the FSH assay they were 24.55 and 17.81%, respectively.

Statistical Analysis

Data collected from birds at PS were evaluated with one-way analysis of variance procedures of SAS® (SAS Institute, 1994). The source of variation for the parameters measured in birds processed at PS was the size groups. The main effects within the 2×3 factorial design, size and feed, were applied to cages in a completely randomized design. Data collected after PS were evaluated by two-way analyses of variance using the General Linear Models procedures of SAS® (SAS Institute, 1994). Sources of variation for initial and final plasma traits, and for carcass parameters at SM were feeding regimen, body size, and the interaction of feed by size. Differences between means were evaluated using Fisher's protected LSD procedure (Peterson, 1985). The error variation for all variables consisted of the variation between birds within the interaction. Means within the interactions were compared only within a feeding regimen.

Plasma hormone profiles were compared using Kolmogorov-Smirnoff curve shape analysis (SAS Institute, 1994). Stepwise regression analysis was used to evaluate the relationship of processing and peak estradiol-17β concentrations with ovarian morphology and carcass parameters using $P < 0.15$ as the limit for inclusion. Pearson correlation coefficients (Steel and Torrie, 1980) were computed between reproductive, carcass, and plasma parameters within the AL and RF feeding regimens. One bird in the RF-LOW group died and as a result SEM values were based in the main effect or interaction group with the fewest birds. Unless otherwise stated, all statements of significance were based on testing at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Reproductive Morphology at Photostimulation

Development of the reproductive tract (oviduct and ovary) at PS was similar on a percentage of BW basis (Table 1). However, on an absolute basis, oviduct weight was lower in LOW birds (0.44 g) than in HIGH birds (1.21 g) and ovary weight was lower in LOW birds (0.59 g) than in STD (0.93 g) and HIGH birds (1.00 g). The weight of the reproductive tracts of STD and HIGH birds indicates that they may be at a more advanced developmental level than in the LOW birds. Both oviduct and ovary weight correlated with BW (oviduct: r = 0.462; $P = 0.010$, ovary: r = 0.512; $P = 0.004$), indicating an effect of body size or BW on reproductive tract development. In birds that consume feed *ad libitum*, evidence of follicular activity can occur as early as 14 wk of age (Hocking *et al.*, 1989), and by 18 wk of age, the ovaries of 25% of *ad libitum* broiler breeders have been reported to contain LYF (Yu *et al.*, 1992a). Carcass lipid stores may also relate to reproductive development, as ovary weight was correlated with fat pad weight in the HIGH body size group (r = 0.837; $P = 0.003$). Renema *et al.* (1999) reported that carcass lipids made up 6.3% of the less reproductively developed, LOW BW birds compared to 10.1% in the HIGH birds.

TABLE 1. Oviduct and ovary weights of LOW, STD, and HIGH BW birds at photostimulation (21 wk of age)

| Parameter | Body size[1] | | | |
	LOW	STD	HIGH	SEM
Oviduct weight				
g	0.44[b]	1.00[ab]	1.21[a]	0.19
% of BW	0.027	0.050	0.051	0.008
Ovary weight				
g	0.59[b]	0.93[a]	1.00[a]	0.08
% of BW	0.036	0.047	0.042	0.004

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

TABLE 2. Oviduct, ovary, and stroma weights at processing in STD, LOW, and HIGH BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

Source	Oviduct		Ovary		Initial ovarian stroma[2]		Bare ovarian stroma[3]	
	Weight	Percentage[1]	Weight	Percentage	Weight	Percentage	Weight	Percentage
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Feed[4]								
AL	62.4	1.86[b]	82.1[a]	2.45[a]	8.63	0.257	3.59[a]	0.106
RF	59.2	2.15[a]	59.4[b]	2.16[b]	7.54	0.274	2.70[b]	0.098
SEM	1.6	0.04	2.8	0.08	0.42	0.013	0.18	0.005
Size[5]								
LOW	61.8	2.16[a]	70.6	2.42	8.58	0.296	3.26	0.112
STD	59.5	1.91[b]	72.3	2.31	7.91	0.255	3.08	0.099
HIGH	61.0	1.93[b]	69.5	2.18	7.78	0.244	3.09	0.095
SEM	1.9	0.05	3.4	0.10	0.52	0.016	0.22	0.007
				Probability				
Source of variation								
Feed	0.16	0.0001	0.0001	0.017	0.069	0.35	0.0008	0.25
Size	0.69	0.002	0.84	0.23	0.50	0.054	0.79	0.16

[a,b]Means within a column and within a source with no common superscript differ significantly.

[1]Percentage = tissue weight/BW × 100.

[2]Initial stroma = ovary without the large yellow follicles (follicles > 10 mm diameter removed).

[3]Bare stroma = initial stroma without the small yellow follicles or large white follicles (follicles > 1 mm diameter removed).

[4]AL = *ad libitum* fed; RF = restricted fed.

[5]STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Reproductive Morphology at Sexual Maturity

The absolute weight of the oviduct at sexual maturity did not differ due to feeding regimen or to body size, averaging 60.8 g (Table 2). As the BW differed between feeding regimens and body size groups (Renema *et al.*, 1999), relative oviduct weights were greater in birds having a lower BW at sexual maturity (Table 2). Ovary weight of AL birds was 38% greater than that of RF birds. This difference was also apparent in the relative ovary weight, which was 2.45 vs 2.16% in AL and RF birds, respectively. Ovary weight did not differ due to body size. Whereas initial stroma weight was numerically greater in AL birds than in RF birds ($P = 0.07$), bare stroma weight of AL birds was higher (3.59 vs 2.70 g for AL and RF, respectively). This difference indicates that overfeeding may have altered ovarian morphology at the level of the prehierarchical follicles. As the bare stroma contained follicles less than 1 mm in diameter, a heavier stroma may indicate greater numbers of these estradiol-17β producing follicles, thereby affecting total estradiol-17β output of these ovaries. Stroma weight relative to BW did not differ due to experimental treatments.

Differences in ovary weight between AL and RF birds were due to the number of LYF (Table 3), which numbered 11.0 and 7.1 in AL and RF birds, respectively. These values concur with those reported by Hocking (1996) for similarly treated hens, but were lower than those of Yu *et al.* (1992a). Yu *et al.* (1992a) found that differences in LYF number similar to those of the current study persisted in some form until 62 wk of age (Yu *et al.*, 1992a). The numbers of SYF, LWF, or MWF were similar in all

treatments. The number of MWF was 186.5 in AL birds compared to 161.0 in RF birds, which, although not significant ($P = 0.07$), supports the premise that the larger bare stroma weight in AL birds may be due to an increased population of small follicles. Small follicle

TABLE 3. Ovarian follicle numbers at processing in STD, LOW, and HIGH BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

Source	Ovarian follicles[1]			
	LYF	SYF	LWF	MWF
		(no.)		
Feed[2]				
AL	11.0[a]	12.6	17.7	186.5
RF	7.1[b]	11.7	16.6	161.0
SEM	0.3	1.2	1.3	9.9
Size[3]				
LOW	8.8	13.3	18.4	174.6
STD	9.5	10.8	17.8	176.1
HIGH	8.9	12.4	15.3	170.5
SEM	0.4	1.5	1.6	12.2
		Probability		
Source of variation				
Feed	0.0001	0.60	0.56	0.070
Size	0.37	0.44	0.36	0.94

[a,b]Means within a column and within a source with no common superscript differ significantly.

[1]LYF = large yellow follicles (> 10 mm diameter); SYF = small yellow follicles (5 to 10 mm diameter); LWF = large white follicles (3 to 5 mm diameter); MWF = medium white follicles (1 to 3 mm diameter).

[2]AL = *ad libitum* fed; RF = restricted fed.

[3]STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

TABLE 4. Ovarian small follicle atresia, unexplained postovulatory follicles (POF), and large yellow follicles (LYF) hierarchy weight and arrangement parameters at processing in STD, LOW, and HIGH BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

| Source | Small atretic follicles[1] | Unexplained POF[2] | LYF parameters | | | |
			F_1 weight	Number in multiple sets[3]	Percentage in multiple sets	Number of hierarchies[4]
	(no.)	——— (g) ———		(no.)	(%)	(no.)
Feed[5]						
AL	10.3[b]	2.13[a]	12.12	8.46[a]	74.2[a]	1.70[a]
RF	32.3[a]	0.31[b]	12.59	2.59[b]	33.7[b]	1.23[b]
SEM	2.7	0.17	0.22	0.48	4.1	0.05
Size[6]						
LOW	26.5	0.86	13.07[a]	5.53	52.8	1.46
STD	20.6	1.40	11.99[b]	6.25	61.0	1.49
HIGH	16.8	1.41	12.00[b]	4.80	48.2	1.44
SEM	3.3	0.21	0.27	0.60	5.1	0.06
			——— Probability ———			
Source of variation						
Feed	0.0001	0.0001	0.13	0.0001	0.0001	0.0001
Size	0.12	0.11	0.007	0.22	0.19	0.83

[a,b]Means within a column and within a source with no common superscript differ significantly.

[1]Number of atretic follicles < 5 mm in diameter.

[2]Postovulatory follicles not accounted for by eggs laid, or by yolks or eggs in oviduct.

[3]Follicles arranged in groups differing by < 1 g.

[4]Hierarchies calculated as LYF divided by positions (groups of follicles within 1 g).

[5]AL = *ad libitum* fed; RF = restricted fed.

[6]STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

numbers were not related to LYF number, concurring with the observations of Hocking *et al.* (1989).

Although small atretic follicles numbered 10.3 in AL birds compared to 32.3 in RF birds (Table 4), the effect of body size was not significant. Whereas the number of small atretic follicles within the AL interaction were similar, values for RF birds were more variable, numbering 42, 31, and 24 for LOW, STD, and HIGH birds, respectively. The number of small atretic follicles in RF birds increased with time to SM (r = 0.619; P = 0.0003). The increased initial stroma weight (r = 0.443; P = 0.016) and bare stroma weight (r = 0.622; P = 0.0003) associated with a longer sexual maturation period suggest that increased rates of small follicle atresia may be utilized to control the small follicle population and ultimately LYF numbers. The inferior egg production of the low-weight birds studied by Robinson and Robinson (1991) may be due in part to elevated rates of small follicle atresia. A naturally high incidence of small follicle atresia may be limiting the ovary's ability to generate an adequate number of LYF to maintain comparable rates of egg production to the heavier bird groups. Within the RF birds of the current study, there was a numerical reduction of 1.2 LYF in LOW compared to STD and HIGH birds.

The incidence of small follicle atresia did not appear to affect LYF numbers of AL birds due to their rapid onset of lay. Renema (1997) theorized that overfeeding during sexual maturation may cause ovarian development to proceed more quickly than the mechanisms controlling excess recruitment of follicles into the LYF hierarchy are able to be established. The problem of excessive LYF

production in rapidly maturing birds could be avoided by extending this period. As the first birds into production are generally the heaviest and fattest at PS, dividing the flock by BW and feeding the heavy group at a lower rate than the light group may be effective in delaying SM and reducing LYF numbers. Dividing pullets according to BW as part of a feed restriction program has been used effectively in broiler breeders to improve flock uniformity at PS (Petitte *et al.*, 1981).

Stepwise regression performed within the AL and RF feeding regimens for the dependent variable, LYF number, revealed that primarily measures of ovarian size are associated with the number of LYF. In AL birds, ovary weight (P < 0.0001) and the proportion of LYF in a multiple arrangement (P = 0.009) were the only significant variables introduced into the model. In RF birds, relative ovary weight (P < 0.0001), small atretic follicle number (P = 0.002), liver weight (P = 0.012), MWF number (P = 0.12), and initial stroma weight (P = 0.10) were included in the model. Liver weight may be an indicator of lipid and yolk precursor synthesis potential in RF birds, where these processes are not hidden by excessive rates of lipid metabolism as in AL birds. The number of small atretic follicles on the ovary were inversely related to LYF number.

The number of unexplained POF was greater in AL (2.13) than in RF (0.31) birds (Table 4). Whereas similar numbers of unexplained POF were present in RF body size groups, the AL-LOW birds (1.5) had numerically fewer POF than AL-HIGH birds (2.6). Unexplained POF numbers have been reported to be higher in birds coming

into production early (Renema *et al.*, 1998), which tend to also have elevated LYF numbers (Hocking *et al.*, 1988). Follicles ovulated prior to first oviposition are presumably lost to processes such as internal ovulation. Melnychuk *et al.* (1997) suggested that increased incidence of unexplained POF in turkeys may be due to the ovary reaching a mature state prior to the oviduct, resulting in loss of potential eggs due to oviduct incompetence. No significant relationships were observed for internal ovulation in the current study, however.

The LOW birds reached SM at the latest age and with the highest egg weight (Renema *et al.*, 1999). Yolk weight (as represented by the weight of the largest LYF) (Table 4) was also greatest in LOW birds. These results concur with expected patterns of increased egg and yolk weight with age. Robinson *et al.* (1996) found that despite significant increases in the weight of the largest follicle in groups of birds reaching SM later, there was no relationship with the weight of the egg. There were no differences between egg characteristics of AL and RF birds.

The arrangement of LYF in the hierarchy of the AL birds was altered due to their elevated LYF numbers. The 3.9 more LYF on AL ovaries (Table 3) resulted in a higher number of follicles being grouped in sets of similar size (8.5 vs 2.6 for AL and RF birds, respectively) (Table 4). Increased pairing of follicles can disrupt optimal ovarian function through increased unsettable egg production (Hocking *et al.*, 1987, 1989; Yu *et al.*, 1992a). The AL birds had 6.5 positions (groups of LYF differing by < 1 g) in the LYF hierarchy compared to 5.8 in the RF birds, indicating that AL ovaries should be able to maintain a higher number of follicles without pairing occurring. However, the AL birds had an average of 1.7 complete hierarchies of LYF on the ovary compared to 1.2 in RF birds. The

hierarchy number represents the average number of follicles that can potentially ovulate on a given day. In 44-wk-old broiler breeders, Robinson *et al.* (1993) reported a 47% difference in birds demonstrating simultaneous follicular development following 2 wk of *ad libitum* or restricted feeding with no affect on LYF number. They concluded that the number of LYF maintained by the ovary may not be as important as the uniformity of spacing of the follicles in ensuring the more regular, single ovulation of follicles.

Plasma Lipid and Hormone Parameters

At PS there was a difference in estradiol-17β concentration due to body size, with HIGH bird plasma estradiol-17β measuring 49 pg/mL compared to 36 pg/mL and 33 pg/mL in the STD and LOW groups, respectively (Table 5). This result suggests that some initial maturation had occurred in the ovaries of HIGH birds prior to PS. Although no follicles over 1 mm in diameter were observed at PS, the greater bare stroma weight of HIGH compared to LOW birds (Table 2) may indicate a larger population of small white follicles (< 1 mm in diameter). The significant correlation between plasma concentrations of FSH and estradiol-17β at PS (r = 0.522; $P < 0.0001$) indicates a relationship between increased FSH and ovary development, as suggested by increased plasma estradiol-17β.

The difference in plasma estradiol-17β concentration between body size groups at SM approached significance ($P = 0.062$) (Table 5), with increased HIGH bird plasma concentrations over STD and LOW bird concentrations. The mean plasma estradiol-17β concentration for the PS to SM time period was identical in AL and RF birds (113 pg/

TABLE 5. Photostimulation (PS), sexual maturity (SM), and mean plasma estradiol-17β (E2), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentration in STD, LOW, and HIGH BW broiler breeders under either *ad libitum* or restricted feed between PS (21 wk of age) and SM

Source	PS hormone concentrations			SM hormone concentrations			Mean hormone concentrations[1]		
	E2	LH	FSH	E2	LH	FSH	E2	LH	FSH
	(pg/mL)	—— (ng/mL) ——		(pg/mL)	—— (ng/mL) ——		(pg/mL)	—— (ng/mL) ——	
Feed[2]									
AL	40.7	2.65	1.36	104.5	3.49	1.64	113.4	4.40[a]	3.47
RF	38.1	2.25	1.25	94.4	3.22	1.70	113.3	3.38[b]	2.85
SEM	3.7	0.23	0.15	7.2	0.40	0.16	7.6	0.13	0.27
Size[3]									
LOW	33.4[b]	2.26	1.20	97.5	2.96	1.56	97.8[b]	3.69	3.21
STD	35.5[b]	2.58	1.28	85.7	3.72	1.73	108.3[b]	4.11	3.30
HIGH	49.1[a]	2.52	1.43	115.1	3.38	1.73	134.1[a]	3.87	2.98
SEM	4.6	0.28	0.18	8.9	0.33	0.19	9.2	0.16	0.33
					Probability				
Source of variation									
Feed	0.58	0.21	0.62	0.32	0.47	0.77	0.99	0.0001	0.11
Size	0.037	0.70	0.67	0.062	0.28	0.77	0.022	0.19	0.79

[a,b]Means within a column and within a source with no common superscript differ significantly.

[1]Mean concentration of plasma hormone in samples taken at 3-d intervals between PS and SM.

[2]AL = *ad libitum* fed; RF = restricted fed.

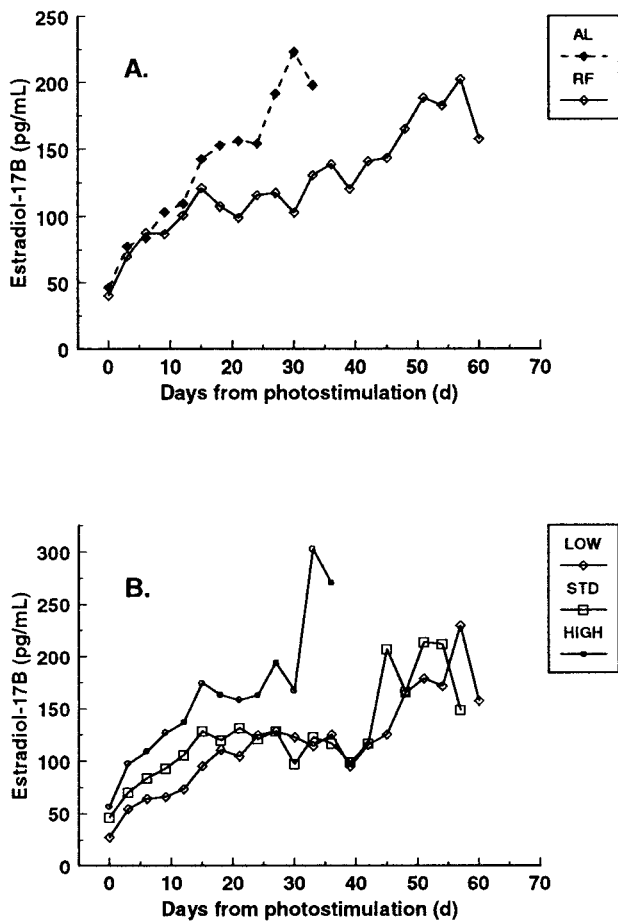[3]STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

FIGURE 1. Plasma estradiol-17β concentration profiles for the main effect, Feed (A) and Size (B) in STD, LOW, and HIGH BW broiler breeders provided either *ad libitum* (AL) or restricted (RF) access to feed beginning at photostimulation (21 wk of age) and ending at sexual maturity. The STD group is the target BW treatment, and the LOW and HIGH curves represent birds naturally 20% lighter or heavier than STD birds, respectively, at photostimulation.

mL), but was greater in the HIGH birds (134 pg/mL) than in the STD and LOW birds (108 and 98 pg/mL, respectively). The plasma estradiol-17β profile of the HIGH birds for the PS to SM period was separated above the STD and LOW curves (Figure 1). The estradiol-17β concentrations were elevated in HIGH birds despite similar small follicle numbers (Table 3) and bare stroma weights (Table 2) to the STD and LOW birds. The steroidogenic capacity of SWF (< 1 mm) have been reported to be enhanced in response to increased feed intake (Yu *et al.*, 1992b). Feed intake for the 2 wk following PS were reported to be lower for LOW than for STD or HIGH birds (Renema *et al.*, 1999). Mean plasma estradiol-17β concentrations increased numerically from LOW to HIGH birds within the RF feeding regimen and were inversely correlated with the number of small atretic follicles on the ovary (r = –0.491; P = 0.008). Elevated mean plasma estradiol-17β concentrations in HIGH birds may be an indicator of the relationship between age at SM and follicular management.

The plasma LH and FSH concentrations at PS and SM did not differ due to feeding treatment or to body size

(Table 5). Mean plasma LH concentration for the PS to SM period was greater in AL birds (4.4 ng/mL) than in RF birds (3.4 ng/mL). This result can be seen clearly in the plasma LH profile (Figure 2): LH concentrations in AL birds were greater than RF birds at 3 to 12 d and 21 to 24 d after PS. The plasma FSH profiles followed a similar pattern in AL and RF birds (Figure 3), although the AL values were only significantly greater at 3 and 6 d after PS. The difference in LH and FSH concentrations between the feeding regimens is purely a feed effect, as birds from these two feeding regimens originated from the same body size groups. The STD body size group had a greater plasma FSH concentration than HIGH birds at 12 and 15 d after PS.

Plasma LH and FSH concentrations increased to peak values typically within 3 d of PS, and subsequently declined as sexual maturation proceeded (Figures 2 and 3), possibly due to negative feedback effects of estradiol-17β on hypothalamic stimulation of LH and FSH release. Vanmontfort *et al.* (1995), using White Leghorn chickens, reported similar LH values and lower FSH values to those
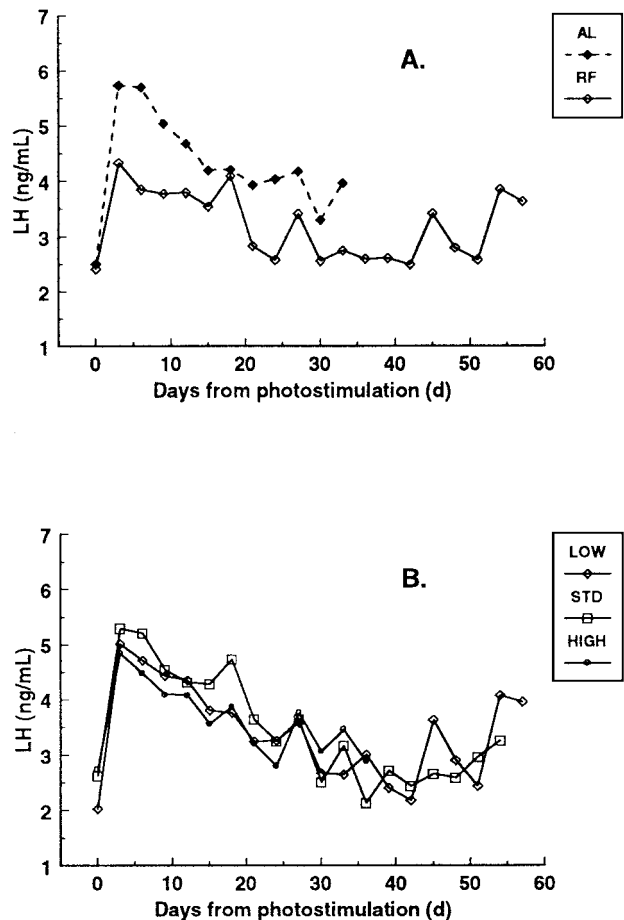

FIGURE 2. Plasma luteinizing hormone (LH) concentration profiles for the main effect, Feed (A) and Size (B) in STD, LOW, and HIGH BW broiler breeders provided either *ad libitum* (AL) or restricted (RF) access to feed beginning at photostimulation (21 wk of age) and ending at sexual maturity. The STD group is the target BW treatment, and the LOW and HIGH curves represent birds naturally 20% lighter or heavier than STD birds, respectively, at photostimulation.
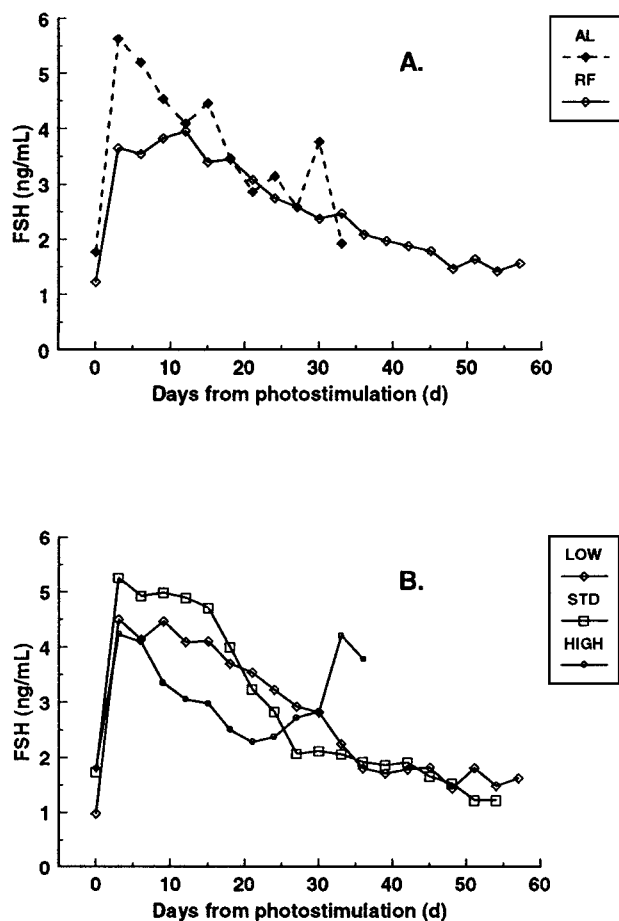
FIGURE 3. Plasma follicle-stimulating hormone (FSH) concentration profiles for the main effect, Feed (A) and Size (B) in STD, LOW, and HIGH BW broiler breeders provided either *ad libitum* (AL) or restricted (RF) access to feed beginning at photostimulation (21 wk of age) and ending at sexual maturity. The STD group is the target BW treatment, and the LOW and HIGH curves represent birds naturally 20% lighter or heavier than STD birds, respectively, at photostimulation.

of the current study, and showed similar declines in LH concentration over time as the plasma steroid concentration rose. In a study by Williams and Harvey (1986), pullets reared on a gradual PS program in which LH concentrations did not change dramatically. As broiler strains have been reported to have higher plasma LH concentrations than egg laying strains (Scanes *et al.*, 1980), only general comparisons can be made with Leghorn data.

The higher plasma concentrations of both LH and FSH in AL compared to RF birds following PS supports existence of a link between nutrition and reproduction. In primates, normal changes in the body's metabolism during the transition from juvenile to mature adult are thought to influence the rate of maturation of the neuroendocrine system and, therefore, the timing of puberty (Steiner, 1987). Blood-borne metabolic factors such as glucose, amino acids, and insulin, were found to augment LH secretion in immature animals. Feed restriction in egg-type hens is known to reduce LH concentrations (Tanabe *et al.*, 1981) which, in mammals, occurs through altered LH-releasing hormone secretion (Steiner,

1987; Cosgrove *et al.*, 1991). As basal levels of factors such as insulin differed between immature and adult animals (Steiner, 1987), energy balance of the animal may also be important in the sexual maturation process. Elevated plasma LH concentrations and reduced sexual maturation time in AL compared to RF birds may be due to processes such as these.

Despite similar mean plasma estradiol-17β values for AL and RF birds between PS and SM, the AL estradiol-17β concentration profile was consistently higher than the RF profile, with significant differences at 18, 21, 27, and 30 d after PS (Figure 1). The rate of change in plasma estradiol-17β concentration between PS and peak estradiol-17β concentration was reduced in the LOW birds compared to the HIGH birds (5.81 vs 9.78 pg/d for LOW and HIGH birds, respectively) (Table 6), indicating a slowed establishment of elevated estradiol-17β concentrations in LOW birds. The estradiol-17β profiles of the feed by size interaction showed a distinct delay in the hormone increase of the RF-LOW birds (Figure 1).

Plasma estradiol-17β concentration in the turkey will typically increase within 4 d of PS, peak just prior to SM, and then decline by about 30% at the onset of egg production (Bacon *et al.*, 1980). In the present experiment, the day of peak estradiol-17β concentration occurred between 5.69 d (RF birds) and 6.63 d (AL birds) prior to SM and was very consistent within each feeding regimen (Table 6). Alignment of the plasma estradiol-17β profiles for each bird with the physiological event of peak estradiol-17β concentration produced similar curves for all treatments (Figure 4), demonstrating that once pubertal ovary development commences, it proceeds at a predictable rate. Kolmogorov-Smirnoff profile shape analysis showed the curves to be similar. The primary difference between the main effects and their interaction was in the length of time for each group prior to substantial increases in estradiol-17β concentration. This delay is particularly apparent in the RF-LOW and RF-STD groups within the feed by size interaction. Both LH and FSH concentrations remain elevated during this delay in ovarian development, which may have affected ovary maturation, as indicated by the differential patterns of ovarian morphology observed at SM.

The peak plasma estradiol-17β concentration of RF birds was greater than that of the AL birds (236.1 vs 185.3 pg/mL for RF and AL birds, respectively) (Table 6). At this time, plasma LH and FSH concentrations were greater in AL than RF birds. Although the higher LH and FSH values may relate to reduced levels of negative steroid feedback in AL birds, the concentrations of both LH and FSH were also greater in AL than in RF bird plasma for up to 15 d prior to peak estradiol-17β concentration.

The significantly reduced peak estradiol-17β concentration in AL birds and numerically reduced values for most other points relative to RF values (Figure 4) has also been observed in growth selected turkey stocks when compared to reproductively selected stocks (Melnychuk *et al.*, 1997). In humans, fatty tissue has been reported to convert estradiol to estriol (Fishman *et al.*, 1975), which can either

TABLE 6. Plasma lipid concentration, time of peak estradiol-17β (E2) concentration, plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations at peak E2, and E2 profile characteristics in STD, LOW, and HIGH BW broiler breeders under either *ad libitum* or restricted feed between photostimulation (PS) (21 wk of age) and sexual maturity (SM)

Source	Plasma lipids at PS	Plasma lipids at SM	Days from PS to peak E2	Days from peak E2 to SM	Peak E2 hormone concentrations[1]			E2 difference		Rate of E2 change to peak
					E2	LH	FSH	PS to SM	PS to peak E2	
	—— (mg/mL) ——		—————— (d) ——————		(pg/mL)	—— (ng/mL) ——		—— (pg/mL) ——		(pg/d)
Feed[2]										
AL	6.38	37.70[a]	18.60[b]	6.63	185.3[b]	4.14[a]	2.64[a]	63.7	144.5[b]	8.19
RF	6.48	20.54[b]	33.78[a]	5.69	236.1[a]	3.61[b]	1.94[b]	56.4	196.9[a]	7.12
SEM	0.14	2.28	1.63	0.48	13.8	0.17	0.23	8.0	13.7	0.78
Size[3]										
LOW	6.60	30.95	34.23[a]	6.04	212.8	3.89	2.15	64.1	179.4	5.81[b]
STD	6.26	24.26	25.50[b]	6.25	197.7	3.98	2.20	50.2	162.1	7.38[ab]
HIGH	6.43	30.66	18.83[c]	6.19	221.5	3.74	2.51	66.0	170.6	9.78[a]
SEM	0.17	2.82	1.97	0.58	16.7	0.20	0.28	9.9	16.7	0.95
					Probability					
Source of variation										
Feed	0.59	0.0001	0.0001	0.16	0.011	0.026	0.035	0.69	0.005	0.33
Size	0.38	0.17	0.0001	0.96	0.59	0.70	0.59	0.45	0.76	0.017

[a–c]Means within a column and within a main effect or the interaction with no common superscript differ significantly.

[1]Concentration of E2, LH, and FSH at time of peak E2 concentration.

[2]AL = *ad libitum* fed; RF = restricted fed.

[3]STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

reduce target tissue response or enhance stimulation through a more biologically active estrogen form. Furthermore, the concentration of the plasma steroid carrier, sex hormone binding globulin, is inversely related to insulin concentration in a BW- and fat content-dependent manner (Botwood *et al.*, 1995) and is reported to be stimulated by estrogen treatment (Toscano *et al.*, 1992). In the current study, the plasma lipid concentrations in AL birds were 84% higher than those of RF birds (Table 6), and AL birds were reported to be fatter (Renema *et al.*, 1999). Increased feed intake in AL compared to RF birds may also affect plasma estradiol-17β concentrations through steroid clearance and recycling mechanisms. Oats, barley chaff, and wheat chaff are excellent binders of estradiol-17β in the gut (Arts *et al.*, 1991), and are utilized in human diets to reduce estrogen exposure. These feed ingredients were all present in some form in the diets fed in the current study (Renema *et al.*, 1999). In sheep, reduced rates of gut passage associated with restricted feeding have been implicated for an increased overall plasma estrogenic activity due to reduced steroid clearance rates and, possibly, to improved hormone resorption (Adams *et al.*, 1994).

Yolk size in RF birds was related to peak plasma estradiol-17β concentration. Peak estradiol-17β concentration correlated with the total weight of the LYF (r = 0.512; *P* = 0.005) and with weights of the individual LYF smaller than the third largest LYF (*P* range of 0.015 to 0.032 for F4 to F8). In a study of the effects of subcutaneous estradiol-17β implants in 59-wk-old broiler breeders, Renema (1997) found that the implants increased ovary weight by 9% over blank implanted birds due to an increase in mean LYF weight. These data suggest that increased estradiol-

17β concentrations stimulate increased rates of yolk synthesis, which results in a larger egg yolk size.

It has previously been shown that birds considerably smaller than the mean BW at PS produce fewer eggs than birds at the target BW or high BW birds due to delayed onset of lay and inferior lipid stores at PS (Robinson and Robinson, 1991). Whereas relative carcass traits of RF birds were reported to be comparable between body size classes at SM (Renema *et al.*, 1999), the number of LYF in the RF-LOW birds was numerically reduced at SM and the number of small atretic follicles significantly increased compared to RF-HIGH birds. As LYF numbers will continue to decline with age, this combination of slightly lower LYF number with increased rates of follicular atresia is likely detrimental to potential egg production. Possible solutions to this problem would be to delay PS of small pullets, or to improve flock BW uniformity at PS to minimize the detrimental effects of small birds on egg production. The use of an AL feeding regimen increased plasma lipid concentrations and ovarian LYF numbers. The inferior LYF arrangement on the AL bird ovaries, as indicated by the high proportion of follicles in a multiple hierarchical arrangement compared to RF bird ovaries (Table 5), concurred with previous studies with overfed birds (Hocking *et al.*, 1987, 1989; Katanbaf *et al.*, 1989; Yu *et al.*, 1992a) and indicated an increased potential for production of unsettable eggs. The AL feeding regimen stimulated increased LH and FSH production compared to RF birds, suggesting that energy balance may interact with, or modulate, BW and age threshold effects in the initiation of SM. The plasma LH and FSH concentrations of RF birds remained elevated for a longer time period than in AL birds following PS. An extended time period in
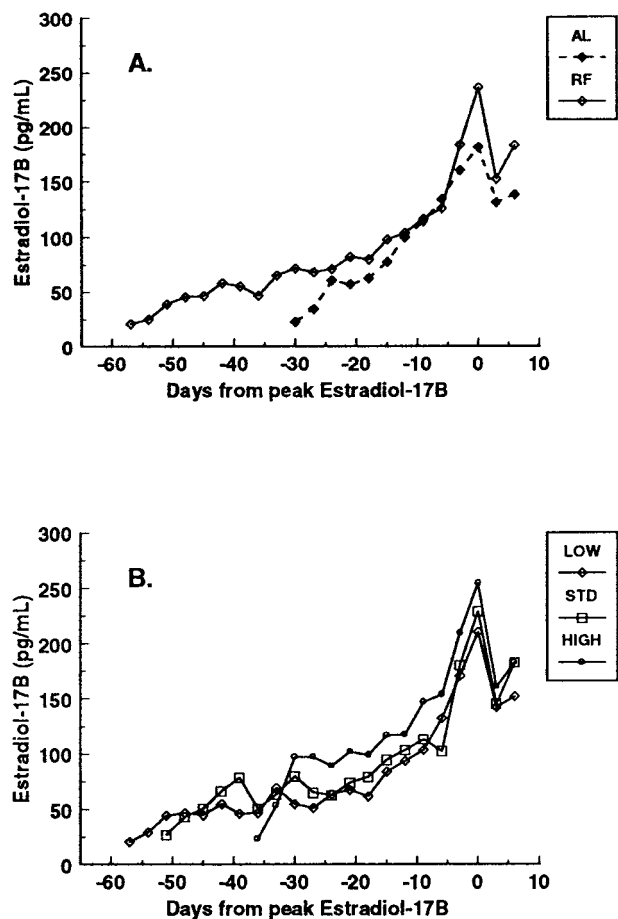
FIGURE 4. Plasma estradiol-17β concentration profiles for the main effect, Feed (A) and Size (B) with the curves adjusted to day of peak Estradiol-17β as time = 0 in STD, LOW, and HIGH BW broiler breeders provided either *ad libitum* (AL) or restricted (RF) access to feed beginning at photostimulation (21 wk of age) and ending at sexual maturity. The STD group is the target BW treatment, and the LOW and HIGH curves represent birds naturally 20% lighter or heavier than STD birds, respectively, at photostimulation.

which the ovary is exposed to elevated LH and FSH concentrations may be essential for the normal development of mechanisms controlling LYF numbers, such as the mechanism of small follicle atresia.

ACKNOWLEDGMENTS

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