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Original Article

Effect of Feed Restriction and Photoperiod on Reproduction and LEPR, MELR mRNA Expression of Layers

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Feed restriction; MELR; Ovary; Photoperiod; Pullet.

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ABSTRACT

Photoperiod and nutrition are major factors that affect the reproductive efficiency particularly in female animals. In this study we examined the interaction of photoperiod and food restriction on growth, sexual maturation and receptor mRNA expressions of leptin, melatonin, and estrogen in abdominal fat and the ovary of pullets. There were no interaction effects between photoperiod and feeding level on body weight, abdominal fat weight, ovary weight at both 14 wk and 18 wk. Abdominal fat weight of feed restriction group was significantly lower compared with the control group at the age of 14 wk, 18 wk, and age of the first egg (AFE) (p<0.05). Ovary *LEPR* (Leptin receptor) gene expression showed an interaction effect of the first egg. Restricted feeding significantly inhibited ovary *ER* (Estrogen receptor), *LEPR* and *MELR1B* (Melatonin 1B receptor) gene expression at 14 wk, 18 wk and the first egg. At 14-week-old, abdominal fat *LEPR* gene expression was significantly lower in long photoperiod group compared with the short photoperiod group. At the first egg, short photoperiod and feed restriction group reduced abdominal fat *LEPR* gene expression. The results indicated that the reproductive activity of pullets is sensitive to feed intake and photoperiod. Feed restriction down regulated the *ER, LEPR*, *MELR1A* (Melatonin 1A receptor) and *MELR1B* mRNA expression of the ovary at 14 wk, 18wk and AFE. Long photoperiod enhanced the *LEPR*, *MELR1A* and *MELR1B* mRNA expression of abdominal fat at AFE.

INTRODUCTION

Laying hens, like many other birds, rely heavily on vision, and light is an important factor within their natural environment (Huber-Eicher *et al.*, 2013). Lighting is the factor that most affects the performance of the production and reproduction of birds, sexual maturation, feeding behavior and productivity of eggs and egg weight (Lewis *et al.*, 2010; Lewis Gous, 2006; Schweanlardner *et al.*, 2012). Nutrition is another major factor that affects the reproductive efficiency particularly in female animals (Brecchia *et al.*, 2006; Walzem Chen, 2014). Various nutritional methods have been employed in breeder pullets for attempting to reduce body weight at the onset of egg laying in order to improve performance during the laying period (Bozkurt *et al.*, 2014; De Coon, 2007; Proudfoot, 1979). Feed restriction has been justified as a means of controlling body weight, improving subsequent reproduction to achieve greater production efficiency without inflicting severe adverse effects on the birds' nutritional requirements (Crouch *et al.*, 2002; De Coon, 2007; Hocking, 2004). Photoperiod cues plays important roles in the regulation of seasonal variations in body mass (BM) and energy balance for many small mammals (Zhao & Wang, 2006).

Leptin, a 146-amino acid protein, is mainly secreted by adipocytes (Paczoska-Eliasiewicz *et al.*, 2006; Sirotkin & Grossmann, 2015) and is

implicated in the regulation of metabolic status, feed intake, reproduction, immune function and body condition in rodent and primates (Bouloumie *et al.*, 1998; Fantuzzi & Faggioni, 2000; Zieba *et al.*, 2005). Gallus Gallus leptin cDNA was first cloned by Taouis (Taouis *et al.*, 1998), which led to a controversy whether a leptin gene exists in the chicken genome (Pitel *et al.*, 2010). Although the sequence of the chicken leptin gene is controversial, cloning of the chicken leptin receptor gene provides evidence of the existence of the leptin homologue in birds (Horev *et al.*, 2000; Liu *et al.*, 2007). Melatonin (N-acetyl-5-methoxytryptamine), an indole hormone, regulates circadian rhythm, hibernation, feeding pattern, thermoregulation, and neuroendocrine function in birds through three different receptor subtypes (*MELR1A*, *MELR1b*, and *MELR1c*) (Adachi *et al.*, 2002; Sinkalu *et al.*, 2015). In mammals, melatonin also influences the reproductive function via activation of receptor sites within the hypothalamic-pituitary-gonadal axis (Malpaux *et al.*, 2001). In birds, melatonin binding sites have been identified in the ovaries, suggesting a possible role of melatonin regulating ovarian functions (Sundaresan *et al.*, 2009).

Following the attainment of minimum age and body weight thresholds, the present study was undertaken to investigate the relationship between photoperiod and feed restriction, and the possible mechanism about how the photoperiod, nutrition or both impact on the adipose store and the sexual maturity in pullets.

MATERIAL AND METHODS

Experimental Design, Birds, and Management

Female Gray Hy-line chicks were purchased from Hebei Huayu Poultry Breeding Company. Chicks were raised according to the management protocols established by Hy-line International. At 10 wk of age, 480 healthy pullets were selected and allotted randomly to one of the 6 treatments, i.e., a 3 (photoperiod: 8L:16D, 12L:12D, or 16L:8D) × 2 (ad libitum or feed restriction) factorial design. The feed restriction was 80% of the ad libitum. The diet contained 11.72 MJ/kg energy, 16.3% crude protein, 0.33% methionine and 0.74% lysine. The specific feeding and photoperiod schedule for the birds were given in Table 1. Each treatment had four replicates comprising 20 pullets each, 4 pullets per cage. Water was provided ad libitum throughout the study. Illumination was provided by 2 15-W compact fluorescent lamps producing a mean illuminance of 15 ± 2.4 lx. Pullets' beaks were trimmed at 7 d of age, and all pullets were wing-banded at 6 wk. The present study was performed in accordance with Hebei Agricultural University Institutional Animal Care and Use Committee Policies for Animal Use under an approved animal.

Table 1 – Feeding and photoperiod treatments.

Groups	Photoperiod	Feeding level
I	8L:16D	Ad libitum*80%
II	8L:16D	Ad libitum
III	12L:12D	Ad libitum*80%
IV	12L:12D	Ad libitum
V	16L:8D	Ad libitum*80%
VI	16L:8D	Ad libitum

Sample Collection

Samples (n = 8 per feeding × photoperiod combination) were collected at the age of 14 wk, 18 wk, and at first egg (AFE), respectively. Body weight was recorded, then pullets were killed by cervical dislocation. Weight of the abdominal fat (including the fat surrounding the gizzard) and the ovaries were measured, and then abdominal fat and ovaries were snap-frozen in liquid nitrogen, and stored at -80° until assayed. Also at the age of 18 wk, 2 pullets from each replicate were selected, weighed, and randomly placed in individual, illuminated, standard laying cages. The age and egg weight at first egg were recorded.

Isolation of Total mRNA and qPCR

Total RNA was isolated from the ovarian cortex and abdominal fat using the RNAeasy mini kit (Omega Bio-Tek, Inc.). Equal amounts of total RNA (1μg) were reverse transcribed into cDNA using the Reverse Transcription kit (TransGen Biotech, Inc). Amplification of specific transcripts was conducted using gene specific primers (Table 2). For each primer pair, only a single product of the predicted size was identified. All amplification products were sequenced to confirm specificity of the reaction. Abundance of specific mRNAs was analyzed by real -time PCR using the 2-ΔΔCt method (Livak Schmittgen, 2001). Values shown for transcript abundance are the Mean ± SEM.

Table 2 – Primer sequences used for qPCR.

Gene symbol	sequence (5'-3')	Product length	Gene ID
LEPR-F	GGCACAAGGTGTTGATTT	118	ENSGALG00000011058
LEPR-R	GATGTCTTCCAGCACTATTT		
MELR1A-F	ATGTTGGTCTTATCTGGGTC	160	ENSGALG00000013576
MELR1A-R	ATGGGAAGTATGAAGTGGAA		
MELR1B-F	ATTCATTTCATCGTCCCTAT	111	ENSGALG00000017228
MELR1B-R	TTTCAGTCTTGGCTTTGTTT		
ER-F	GCTCAAGAAGAGAACGCT	194	ENSGALG00000011801
ER-R	AGGACGACTCACCAACAC		
β-*actin-F*	TATGTGCAAGGCCGGTTTC	110	NM_205518.1
β-*actin-R*	TGTCTTTCTGGCCCATACCAA		

Statistical Analysis

The data were analyzed by two factors analyses of variance using the General Linear Models procedures of SPSS. When significant differences were determined for the main effects, comparison among means were made using the Duncan procedure. Unless otherwise stated, all statements of significance were assessed using $p<0.05$.

RESULTS

Body Weight, Ovary Weight and Abdominal Fat Weight

There were no interaction effects between photoperiod and feeding level on body weight, abdominal fat weight, and ovary weight at 14 wk, 18 wk, and at first egg (Table 3). However, treatment effects were detected. Abdominal fat weight of pullets from the feed restricted group was significantly lower compared with pullets from the ad libitum group at the age of 14wk, and AFE (*p<0.05*). Pullets' ovary weight in the feed restricted group was lower compared to the ones in ad libitum group at the age of 14 wk ($p<0.05$). Lighting program effects were found on ovary weight at AFE only, it was lower in the 16L:8D group compared with the 12L:12D group but not the group of 8L:16D (*p<0.01*).

Age of First Egg and First Egg Weight

There were no interaction effects between photoperiod and feed restriction on the age of the first egg and first egg weight (Table 4). However, long photoperiod significantly reduced the age of the first egg compared with the short photoperiod (*p<0.05*). The average age at the first egg was 146 d for the

Table 4 – Effect of food restriction and photoperiod on age of first egg, and first egg weight.

Group	Age of First Egg/d	First Egg Weight/g
8L:16D*Restriction	156.45±8.42^{Aab}	48.91±3.51
8L:16D*Ad libitum	159.80±15.77^{Aa}	48.87±3.87
12L:12D*Restriction	153.77±18.19^{Aab}	46.00±4.97
12L:12D*Ad libitum	147.87±11.36^{ABb}	47.33±5.74
16L:8D*Restriction	153.14±12.22^{Aab}	47.71±5.86
16L:8D*Ad libitum	139.33±6.76^{Bc}	46.40±6.33
8L:16D	158.38±13.06^{Aa}	48.88±3.65
12L:12D	150.60±14.93^{ABb}	46.71±5.34
16L:8D	146.00±11.90^{Bb}	47.03±6.04
Restriction	154.32±13.45	47.47±4.98
Ad libitum	149.00±14.38	47.53±5.39
Photoperiod *p*-value	0.004	0.264
Feed level *p*-value	0.057	0.995
Photoperiod*Feed level *p*-value	0.053	0.635

Table 3 – Effect of feeding level and photoperiod on body weight, abdominal fat weight and ovary weight at the age of 14wk, 18wk, and AFE.

Group	14 wk			18 wk			AFE		
	Body weight/g	Abdominal fat weight/g	Ovary weight/g	Body weight/g	Abdominal fat weight/g	Ovary weight/g	Body weight/g	Abdominal fat weight/g	Ovary weight/g
8L:16D*Restriction	930.00±130.62	2.95±2.16	0.36±0.11	1108.60±80.48	8.50±4.30^{ab}	0.58±0.06	1451.20±90.69^{ab}	25.49±9.52^{b}	28.85±6.60^{b}
8L:16D*Ad libitum	1009.00±44.92	5.99±4.96	0.47±0.10	1179.00±109.42	17.09±14.97^{a}	0.57±0.07	1563.28±75.88^{a}	37.47±8.25^{a}	40.98±8.23^{a}
12L:12D*Restriction	896.67±124.53	2.44±1.84	0.34±0.07	1093.33±64.73	3.98±1.72^{b}	0.62±0.12	1360.20±273.20^{b}	23.32±3.93^{b}	43.62±9.62^{ab}
12L:12D*Ad libitum	1005.00±87.11	6.11±6.69	0.50±0.11	1131.60±161.15	11.39±6.95^{ab}	0.67±0.22	1467.28±75.75^{ab}	32.36±12.31^{ab}	39.48±5.01^{a}
16L:8D*Restriction	932.50±127.05	2.40±1.00	0.38±0.04	1143.25±124.16	5.89±4.16^{ab}	0.70±0.17	1433.75±32.45^{ab}	24.82±10.47^{b}	37.66±4.90^{ab}
16L:8D*Ad libitum	981.67±88.08	8.25±5.13	0.39±0.04	1164.67±55.19	11.13±11.38^{ab}	0.57±0.16	1455.87±106.99^{ab}	35.08±9.34^{ab}	17.91±16.38^{b}
8L:16D	969.50±101.06	4.47±3.94	0.42±0.11	1143.80±97.86	12.80±11.33	0.58±0.06	1507.24±98.51	31.48±10.51	34.91±9.50^{AB}
12L:12D	964.38±109.13	4.74±5.49	0.44±0.12	1117.25±128.17	8.61±6.57	0.65±0.18	1427.13±166.36	28.97±10.63	41.03±6.74^{A}
16L:8D	953.57±106.53	4.91±4.36	0.38±0.03	1152.43±94.10	8.14±7.72	0.64±0.17	1443.23±66.95	29.21±10.68	29.20±14.59^{B}
Restriction	922.50±116.92	2.64±1.63^{a}	0.36±0.07^{a}	1116.33±88.07	6.50±3.96	0.63±0.12	1422.63±135.38	24.72±8.15^{a}	35.48±8.89
Ad libitum	1001.15±67.98	6.56±5.33^{b}	0.46±0.10^{b}	1157.46±116.79	13.52±11.00	0.61±0.15	1501.57±91.32	34.95±9.65^{b}	35.08±13.11
Photoperiod *p*-value	0.928	0.888	0.701	0.761	0.440	0.557	0.250	0.741	0.020
Feed level *p*-value	0.078	0.030	0.026	0.358	0.066	0.548	0.107	0.017	0.269
Photoperiod*Feed level *p*-value	0.864	0.804	0.300	0.901	0.929	0.515	0.705	0.951	0.004

In the same row, values with no letter or the same letter superscripts mean no significant difference ($p>0.05$), while with different small letter superscripts mean significant difference ($p<0.05$), and with different capital letter superscripts mean significant difference ($p<0.01$). The same as below.

16L:8D group and 156.45 d for the 8L:16D group. Photoperiod or feed restriction did not significantly affect the first egg weight (*p>0.05*).

LEPR, MELR1A, MELR1B Gene Expression

There were no photoperiod and feeding level interaction effects on abdominal fat *LEPR*, *MELR1A*, and *MELR1B* gene expression at 14 wk and 18 wk except *LEPR* at AFE (Table 5). At 14-week-old, abdominal fat *LEPR* and *MELRIA* gene expressions were significantly lower in the 16L:8D photoperiod group compared with the 8L:16D photoperiod group (*p<0.05*); feed restriction increased the abdominal fat *LEPR* expression compared with the ad libitum group at the age of 14 wk (*p<0.05*). However, at first egg, both LEPR and MELRIA gene expressions were higher in the 16L:8D long photoperiod group than in the 8L:16D short photoperiod (Table 5).

Table 5 – Effect of food restriction and photoperiod on *LEPR*, *MELR1A*, *MELR1B* gene expression in abdominal fat of pullets.

Group	14 wk			18 wk			AFE		
	LEPR	*MELR1A*	*MELR1B*	*LEPR*	*MELR1A*	*MELR1B*	*LEPR*	*MELR1A*	*MELR1B*
8L:16D*Restriction	14.33±0.92^{c}	14.15±1.09^{b}	12.92±0.89	12.85±0.32^{ab}	12.08±1.67	11.09±1.99	10.91±1.91^{a}	12.42±2.37^{ab}	10.11±2.65
8L:16D*Ad libitum	13.63±0.62^{abc}	13.81±0.94^{b}	12.95±0.61	11.32±4.84^{ab}	11.16±4.53	10.31±5.11	13.73±1.23^{b}	10.74±0.66a	10.21±0.67
12L:12D*Restriction	14.02±0.24^{bc}	14.35±1.40^{b}	13.59±2.18	14.27±1.13^{b}	14.35±1.15	13.21±1.06	14.36±0.60^{b}	14.31±1.86^{b}	12.78±2.39
12L:12D*Ad libitum	13.02±0.84^{ab}	13.06±1.17^{ab}	12.65±2.15	14.10±0.62^{b}	12.21±2.41	12.33±2.65	13.32±0.88^{b}	13.09±2.98^{ab}	12.17±1.74
16L:8D*Restriction	13.12±0.80^{ab}	12.16±0.96^{a}	13.23±1.72	12.02±0.80^{ab}	11.92±1.11	11.60±0.77	13.53±1.06^{b}	13.96±1.^{02b}	10.97±2.44
16L:8D*Ad libitum	12.84±0.92^{a}	12.84±0.28^{ab}	13.81±1.69	10.94±2.76^{a}	12.65±3.12	12.28±2.36	14.42±2.01^{b}	13.86±1.34^{b}	11.30±1.72
8L:16D	14.01±0.84^{a}	13.99±0.99^{a}	12.94±0.74	12.08±3.37	11.2±3.29	10.70±3.72	12.32±2.12^{a}	11.58±1.87^{A}	10.16±1.84^{a}
12L:12D	13.48±0.81	13.65±1.39^{a}	13.08±2.11	14.19±0.87	13.28±2.12	12.77±1.98	13.90±0.88^{b}	13.70±2.43^{B}	12.47±2.00^{b}
16L:8D	12.99±0.81^{b}	12.46±0.79^{b}	13.49±1.62	11.48±2.02	12.28±2.27	11.94±1.71	13.94±1.55^{b}	13.91±1.11^{B}	11.12±2.05^{ab}
Restriction	13.86±0.87^{a}	13.59±1.47	13.22±1.55	13.05±1.26	12.78±1.70	11.97±1.59	12.85±1.97	13.52±1.91	11.20±2.59
Ad libitum	13.17±0.81^{b}	13.25±0.97	13.06±1.61	12.12±3.37	12.01±3.32	11.64±3.51	13.85±1.44	12.45±2.23	11.16±1.57
Photoperiod P-value	0.027	0.013	0.735	0.022	0.310	0.190	0.016	0.000	0.034
Feed level P-value	0.025	0.421	0.860	0.249	0.385	0.719	0.088	0.297	0.910
Photoperiod*Feed level *p*-value	0.585	0.141	0.591	0.774	0.416	0.736	0.016	0.341	0.965

There were also no photoperiod and feeding level interaction effects on the ovary *ER*, LEPR, *MELR1A* and *MELR1B* gene expression at 14 wk, 18 wk and first egg (Table 6). Feed Restriction significantly inhibited ovary *ER*, *LEPR, MEIRIB,* and *MELR1B* gene expression at 14 wk, 18 wk, and first egg, except MEIRIB at 18 wk . Photoperiod did not show significant differences on all measured genes at all the examined time periods (Table 6).

DISCUSSION

It has been suggested that there is a BW or body composition threshold for the onset of sexual maturation (Brody *et al.*, 1980; Brody *et al.*, 1984). Chen (2007) reported that all lighting programs were effectively able to stimulate the sexual maturation process, however, photoperiod had no effect on BW or absolute abdominal fat weight at first egg. Results from the current study showed that photoperiod had no effect on BW and absolute abdominal fat, but the 16L:8D photoperiod group reduced ovary weight in chickens at first egg compared with the 12L:12D group. Feed restriction early in life has been proposed as a strategy for improving feed efficiency and reducing body fat in broilers (Akande Atteh, 2016; Xu *et al.*, 2017). Previous studies have shown that feed restriction (75% of control ad libitum) delayed the broilers age of sexual maturity and significantly reduced ovary weight, number of yellow follicles, number of atretic yellow follicle, incidence of double hierarchy, internal ovulation as compared to control from 19 to 25 wk of age (Madnurkar *et al.*, 2014). During egg production, feed restriction resulted in significantly lower body and abdominal fat pad weights compared with unrestricted feeding (Richards *et al.*, 2003). The data of the present study showed that feed restriction could reduce abdominal fat weight at the age of 14 wk and first egg, and there was an interaction effect between photoperiod and feed restriction on ovary weight at first egg. The age at first egg (AFE) was affected in a curviform by the lighting intensity and length of the photoperiod (Lewis *et al.*, 1997). Exposure to photoperiods of 17L:7D, 15L:9D, 13L:11D or 11L:13D significantly affected the age at first egg (Chen *et al.*, 2007). The average age at first egg was 144.8 d for the 17L:7D group and 150.5 d for the 11L:13D group. In the present study, the age of the first egg in the 16L:8D photoperiod group was 10.45 d earlier than in the 8L:16D photoperiod group.

Table 6 – Effect of food restriction and photoperiod on ER, *LEPR*, *MELR1A*, *MELR1B* gene expression in ovary of pullets.

Group	14 wk				18 wk				AFE			
	ER	*LEPR*	*MELR1A*	*MELR1B*	*ER*	*LEPR*	*MELR1A*	*MELR1B*	*ER*	*LEPR*	*MELR1A*	*MELR1B*
8L:16D*Restriction	3.70±1.42^{A}	0.54±0.31^{A}	14.87±8.82	0.52±0.23^{A}	3.97±1.88^{B}	0.68±0.13^{A}	13.99±2.71^{a}	0.63±0.18^{A}	2.12±0.68^{A}	0.20±0.05^{A}	5.18±1.87^{a}	0.34±0.13^{A}
8L:16D*Ad libitum	9.13±0.61^{B}	11.81±0.38^{B}	8.14±0.58	13.59±0.71^{B}	10.57±0.53^{C}	13.15±0.37^{C}	9.13±1.11	14.21±1.06^{B}	11.47±1.32^{B}	13.90±0.15^{B}	8.57±1.08^{b}	12.95±1.14^{B}
12L:12D*Restriction	3.46±1.21^{A}	0.54±0.10^{A}	11.05±4.53	0.36±0.16^{A}	2.76±1.29^{AB}	0.54±0.17^{A}	13.33±14.29	0.49±0.58^{A}	2.01±0.86^{A}	0.30±0.11^{A}	6.63±3.76	0.25±0.10^{A}
12L:12D*Ad libitum	9.59±0.32^{B}	11.87±0.40^{B}	8.20±0.43	12.88±0.73^{B}	10.38±0.59^{C}	12.64±0.09^{B}	9.35±2.68	13.83±0.47^{B}	10.87±1.01^{B}	13.65±0.37^{B}	7.71±0.42	12.95±0.63^{B}
16L:8D*Restriction	2.78±0.99^{A}	0.55±0.27^{A}	14.18±7.74	0.51±0.57^{A}	1.76±0.25^{A}	0.38±0.07^{A}	4.52±1.43^{b}	0.30±0.27^{A}	1.92±0.70^{A}	0.34±0.17^{A}	6.36±1.97	0.23±0.17^{A}
16L:8D*Ad libitum	9.25±0.45^{B}	12.34±0.83^{B}	8.13±0.57	13.54±0.35^{B}	9.68±0.28^{C}	13.06±0.50^{BC}	9.28±1.52	13.58±1.10^{B}	11.81±0.49^{B}	13.59±0.55^{B}	8.01±0.95	12.61±1.08^{B}
8L:16D	6.42±3.02	6.18±5.89	11.50±6.92	7.06±6.85	6.45±3.71	6.91±6.57	11.56±3.22	7.42±7.19	6.37±4.98	6.43±7.16	6.72±2.31	6.07±6.63
12L:12D	6.52±3.31	6.21±5.92	9.63±3.41	6.62±6.56	6.57±4.13	6.59±6.38	11.34±9.92	7.16±7.05	5.94±4.75	6.24±7.04	7.11±2.73	5.89±6.71
16L:8D	6.02±3.46	6.44±6.18	11.16±6.11	7.02±6.82	5.72±4.18	6.72±6.69	6.90±2.86	6.94±7.04	6.31±5.25	6.23±6.99	7.09±1.75	5.73±6.56
Restriction	3.31±1.21^{A}	0.55±0.23^{A}	13.37±7.03^{A}	0.46±0.35^{A}	2.83±1.54^{A}	0.54±0.17^{A}	10.61±9.00	0.47±0.38^{A}	2.02±0.70^{A}	0.27±0.13^{A}	6.00±2.53^{a}	0.28±0.14^{A}
Ad libitum	9.32±0.49^{B}	12.01±0.59^{B}	8.16±0.50^{B}	13.34±0.67^{B}	10.16±0.59^{B}	12.95±0.41^{B}	9.25±1.75	13.87±0.89^{B}	11.39±1.02^{B}	13.73±0.37^{B}	8.13±0.90^{b}	12.84±0.92^{B}
Photoperiod P-value	0.379	0.282	0.641	0.084	0.882	0.994	0.189	0.989	0.553	0.747	0.929	0.739
Feed level P-value	0.000	0.000	0.005	0.000	0.000	0.000	0.577	0.000	0.000	0.000	0.015	0.000
Photoperiod*Feed level *p*-value	0.382	0.302	0.619	0.344	0.409	0.078	0.216	0.897	0.481	0.147	0.437	0.869

There was no interaction effect between photoperiod and food restriction on the age of the first egg and first egg weight. The current data further evidences that the photoperiod remains the primary mediator of regulating AFE in birds.

A study of Japanese quail in which leptin was injected in ovo enhanced the growth rate during embryonic and postembryonic development and led to earlier hatching and puberty (Lamosova *et al.*, 2003). Furthermore, whereas a single injection of leptin in chickens resulted in attenuation of feed intake (Cassy *et al.*, 2004; Denbow *et al.*, 2000; Lohmus *et al.*, 2003; Raver *et al.*, 1998; Taouis *et al.*, 2001), but not chronic leptin injections lasting several weeks. It is now clear that reproductive maturation will not take place in the complete absence of leptin signaling (i.e. in mammals lacking either functional leptin or its receptor) but leptin is not necessarily the rate-limiting determinant for puberty onset, it acts rather as a permissive factor or 'metabolic gate' (Foster Nagatani, 1999). For example, leptin will not advance the timing of normal puberty in ad libitum fed rats, but in moderately feed restricted prepubertal rats, puberty is delayed. This delay can be prevented by simultaneous treatment with leptin, leading to the result that puberty occurs at a similar time to ad-libitum fed rats (Cheung *et al.*, 1997). This study suggested that feed restriction significantly inhibited ovary *LEPR* gene expression at 14 wk, 18 wk and at first egg, but did not significantly affect abdominal fat *LEPR* gene expression at 18 wk and at first egg. The current data evidences that photoperiod mainly mediates the abdominal fat *LEPR* gene expression, while feed restriction mostly mediated the ovary *LEPR* gene expression.

In birds, melatonin binding sites have been identified in the ovaries, suggesting a possible role of melatonin regulating ovarian functions (Sundaresan *et al.*, 2009). The present findings are in line with the hypothesis that melatonin directly acts on the gonads (Ayre Pang, 1994). In the current study, we observed two main subtypes of melatonin receptors expression in the ovary. The differential distribution of *MELR1A* and *MELR1B* in ovarian tissues suggests that these receptors mediate distinct downstream cellular functions of melatonin in these tissues. There was a trend towards feed restriction reducing ovary expression of *LEPR* and *MELR1B* mRNA in treated chicken.

The role of estrogens in hen reproduction has been well established (Hrabia *et al.*, 2008). Therefore, the mRNA expression of estrogen receptors under different photoperiod and feed restriction was examined within

the ovaries of pullets. The current study showed that there was a trend towards feed restriction reducing ovary expression of *ER* mRNA in treated chickens, but photoperiod did not affect *ER* mRNA expression.

CONCLUSION

Taken together, the results of this study suggest that feed restriction down regulated the *ER, LEPR*, *MELR1A* and *MELR1B* mRNA expression of the ovary at 14 wk, 18 wk, and AFE. Long photoperiod enhanced the *LEPR*, *MELR1A* and *MELR1B* mRNA expression of abdominal fat at AFE. Moreover, a better understanding of the mechanisms governing the partitioning of leptin and melatonin between adipose and ovarian tissue were reached, thereby enabling strategies to effectively control the threshold of sexual maturation in chickens.

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