Productivity, reproductive performance, and fat deposition of laying duck breeders in response to concentrations of dietary energy and protein

W. G. Xia,[*,2] K. F. M. Abouelezz,[*,†,2] A. M. Fouad,[*,‡,2] W. Chen,[*] D. Ruan,[*] S. Wang,[*]
M. M. M. Azzam,[*,§] X. Luo,[*] Q. L. Fan,[*] Y. N. Zhang,[*] and C. T. Zheng[*,1]

*Institute of Animal Science, Guangdong Academy of Agricultural Sciences, State Key Laboratory of Livestock and Poultry Breeding, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Key Laboratory of Poultry Genetics and Breeding, Ministry of Agriculture, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangdong Public Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, P. R. China; †Department of Poultry Production, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt; ‡Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt; and §Poultry Production Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt

ABSTRACT This study evaluated the optimal concentrations of dietary ME and CP for the productivity, reproductive performance, and fat deposition in laying duck breeders aged from 29 to 45 wk. Using a 3 × 3 factorial arrangement of treatments, 648 Longyan laying duck breeders with similar BW were randomly assigned to experimental diets of 2,600 (H_{ME} = high ME), 2,500 (M_{ME} = medium ME), or 2,400 (L_{ME} = low ME) kcal of ME/kg, each containing 19% (H_{CP} = high CP), 18% (M_{CP} = medium CP), or 17% (L_{CP} = low CP) CP. Each dietary treatment contained 6 replicates of 12 birds each. Compared with birds fed the L_{CP} diet, the egg production and egg mass were higher in birds fed H_{CP} and M_{CP} ($P < 0.01$), with better feed conversion ($P < 0.01$). Interactions were detected between ME and CP levels in egg production, egg mass, and feed conversion ratio as the $L_{ME}M_{CP}$ diet was the best ($P < 0.05$). The birds fed M_{ME} ($P < 0.05$) had the lowest abdominal fat. The percentage of healthy ducklings was affected by maternal dietary CP ($P < 0.05$) with the H_{CP} being the highest. The weight of large yellow follicles/ovarian weight was higher in birds fed H_{CP} and M_{CP} ($P < 0.05$), whereas the weight of small yellow follicles/ovarian weight was higher in birds fed H_{CP} and L_{CP} ($P < 0.05$). The hepatic transcript abundances of genes for very low density apolipoprotein-II and carnitine palmitoyltransferase-1A (*CPT-1A*) were lowest in birds fed M_{CP}, whereas the highest abundance of *CPT-1A* transcripts was found in birds fed M_{ME}. These results revealed that the diets containing ME of 12.9 kcal/g protein optimized both egg production and egg mass, while the feed conversion was optimized at 12.8 kcal ME/g protein. Using ME to CP ratio of 12.9 kcal/g protein, i.e., 2,451 kcal ME/kg at 19% CP, maximized the reproductive performance and hatchling outcome of Longyan laying duck breeders.

Key words: metabolizable energy, crude protein, laying duck breeder

2019 Poultry Science 0:1–10
http://dx.doi.org/10.3382/ps/pez061

INTRODUCTION

The levels of dietary energy and protein required to maintain health and productivity in laying hens, broiler breeders, and pigeons have been studied widely (Novak et al., 2008; Moraes et al., 2014; Bu et al., 2015), but little information is available regarding laying ducks. The NRC (1994) and MAFF Japan (1992) summarized the dietary energy and protein requirements for Pekin ducks, which would not meet the needs of laying duck breeders in China due to the latter's greater genetic capacity for egg production, size, quality, fertility, and hatchability. It is necessary, therefore, to determine the optimal dietary ME and CP levels in laying duck breeders in order to maximize their productivity. A previous study with guinea fowl indicated that a diet containing 2,750 kcal ME/kg and 18% CP improved egg number, cost/dozen eggs, and livability (Oke et al., 2003). The results of Li et al. (2015) suggested that moderate ME (2,400 kcal/kg) and 16% protein with a balanced amino acid profile are very important for optimizing the feed conversion ratio, egg production, and egg mass in Lohmann Brown laying hens. Thongwittaya and Tasaki (1992) found that the lowest feed cost required to produce 1 kg eggs was reached with

Received April 11, 2018.
Accepted February 14, 2019.
[1]Corresponding author: zhengcht@163.com
[2]These authors contributed equally to this study.

Table 1. Composition and nutrient levels in the experimental diets (%, as fed basis).

Item[1]	H_{ME}			M_{ME}			L_{ME}		
	H_{CP}	M_{CP}	L_{CP}	H_{CP}	M_{CP}	L_{CP}	H_{CP}	M_{CP}	L_{CP}
Ingredient									
Corn	51.3	54.0	56.8	46.8	49.6	52.3	42.4	45.1	47.9
Soybean meal	16.2	17.2	18.1	15.7	16.7	17.6	15.2	16.2	17.1
Wheat middling	10.9	9.60	8.35	16.3	15.0	13.8	21.7	20.4	19.2
Corn gluten meal	10.2	7.70	5.32	9.72	7.30	4.88	9.27	6.85	4.43
Limestone	8.74	8.70	8.70	8.80	8.77	8.75	8.86	8.84	8.81
Di-calcium phosphate	1.32	1.35	1.37	1.26	1.28	1.30	1.19	1.21	1.23
DL-Methionine	0.03	0.06	0.10	0.03	0.07	0.10	0.04	0.07	0.10
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100	100	100	100
Analysis results[3]									
DM, %	88.3	87.8	88.5	88.1	88.8	88.5	88.6	88.9	88.7
Gross energy, kcal/kg	3,535	3,547	3,538	3,529	3,527	3,525	3,502	3,493	3,496
CP, %	19.4	18.0	16.8	19.3	18.5	17.6	19.5	18.8	17.6
Calculated results[4]									
ME, kcal/kg	2,600	2,600	2,600	2,500	2,500	2,500	2,400	2,400	2,400
CP, %	19.0	18.0	17.0	19.0	18.0	17.0	19.0	18.0	17.0
Ca, %	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60
Available P, %	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Total Lys, %	0.90	0.85	0.80	0.90	0.85	0.80	0.90	0.85	0.80
Total Met + Cys, %	0.74	0.70	0.66	0.74	0.70	0.66	0.74	0.70	0.66
ME:CP, kcal/g	13.7	14.4	15.3	13.2	13.9	14.7	12.6	13.3	14.1

[1]H_{CP} = high CP (19%); M_{CP} = medium CP (18%); L_{CP} = low CP (17%); H_{ME} = high ME (2,600 kcal/kg); M_{ME} = medium ME (2,500 kcal/kg); L_{ME} = low ME (2,400 kcal/kg).

[2]The premix provided the following per kilogram of diet: vitamin A 12,000 IU, vitamin D_3 1,800 IU, vitamin E 26 IU, vitamin K 1.0 mg, vitamin B_1 3.0 mg, vitamin B_2 9.6 mg, vitamin B_6 6.0 mg, vitamin B_{12} 0.03 mg, choline 500 mg, D-calcium pantothenate 28.5 mg, folic acid 0.6 mg, biotin 0.15 mg, Fe 50 mg, Cu 10 mg, Mn 90 mg, Zn 90 mg, I 0.50 mg, and Se 0.40 mg.

[3]Analyzed in triplicates.

[4]According to Ministry of Agriculture of China (2004).

2,700 kcal ME/kg and 16.5% CP diets compared with higher ME and lower protein diets in Khaki Campbell × Thai native laying ducks. In quail breeders, Tarasewicz et al. (2006) found that a dietary CP level greater than 19% increased the number of eggs laid, and the dietary CP did not exert any effect on the final BW.

The laying Longyan ducks are originally descended from crossing between the Longyan breed, Putian White breed, and Putian Black breed (Lin et al., 2014). They are popular egg layers in South China of approximately 1.5 kg BW and 280 to 300 annual egg production (Xia et al., 2015). As part of a systematic program for optimizing the performance of the highly productive laying duck breeders, the current study aimed to evaluate the optimal dietary ME and CP for the productivity, reproductive performance, and fat deposition in Longyan duck breeders.

MATERIALS AND METHODS

Animals and Treatments

All of the procedures employed in this study were approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences. In total, 648 Longyan laying duck breeders having the same genetic background in terms of the parental generation and the same average BW at an age of 29 wk were randomly assigned to 9 treatments in a 3 × 3 factorial arrangement. The ducks fed experimental diets of 2,600 ($\mathbf{H_{ME}}$ = high ME), 2,500 ($\mathbf{M_{ME}}$ = medium ME), or 2,400 ($\mathbf{L_{ME}}$ = low ME) kcal of ME/kg, each containing 19% ($\mathbf{H_{CP}}$ = high CP), 18% ($\mathbf{M_{CP}}$ = medium CP), or 17% ($\mathbf{L_{CP}}$ = low CP) CP (Table 1). The different experimental diets were offered to the birds between 29th and 45th wk of age, and the data collection period lasted 16 wk. A total of 6 replicates, each of 12 birds, were assigned for each dietary treatment. All birds were individually housed in galvanized steel battery cages (length 27.8 cm × width 40 cm × height 55 cm, Guangzhou Huanan Poultry Equipment Co., Ltd, Guangzhou, China) equipped with a nipple drinker and feeder in a rearing house. The total sulfur amino acids and lysine contents in the tested diets were maintained at a ratio of 0.82. The dietary ME values were calculated according to the values of feed ingredients for chickens (Ministry of Agriculture of China, 2004). The other dietary nutrient levels were set as described by Xia et al. (2017). Fresh drinking water was available ad libitum throughout, whereas 80.5 g as fixed feed amount was introduced twice daily at 7:00 am and 3:00 pm to provide 386, 402, and 419 kcal ME intake/d and 27.4, 29.0, and 30.4 g CP intake/d. In addition to ambient daylight, 4 h of artificial light (incandescent lighting at 15 lx) was provided from 6:30 pm to 10:30 pm to give a light: dark regime of 16: 8 h.

Tissue Sampling and Storage

At completion of treatments at 45 wk of age, 2 birds were selected randomly from each replicate and blood samples were collected from the left wing vein in 5 mL vacutainers at 10:00 am after an overnight fast for 12 h. After 30 min, the plasma was separated by centrifugation (1,200 × g for 10 min) and stored in 0.5 mL Eppendorf tubes at –20°C. The birds were then killed and exsanguinated, and the ovaries, oviducts, abdominal fat, and livers were collected. Samples from the liver were rinsed quickly with phosphate-buffered saline, snap-frozen in liquid nitrogen, and stored at –80°C.

Productivity Performance

During the laying period, the numbers of total, broken, and shell-less eggs were recorded daily on a replicate basis. All of the eggs produced were weighed individually and graded daily (European Economic Community, 1989). Feed intake, egg production, egg weight, egg mass (egg weight/bird/d), and feed conversion ratio (feed intake/egg weight) were calculated daily on a per replicate basis, and then expressed as averages for the 16-wk period from 29 to 45 wk of age.

BW and Abdominal Fat

The initial and final live BWs of birds were recorded at 29 and 45 wk of age on a replicate basis, and the BW loss of birds was calculated. Abdominal fat collected from birds fasted for 12 h prior to dissection was weighed using an electronic balance (AB-S/PH, Mettler Toledo, Switzerland) and expressed as percentage of live BW.

Incubation Indices

During the peak laying period, all birds were inseminated artificially twice within the 41st wk with 100 μL of diluted fresh semen (diluted 1: 1 v/v with 0.9% saline solution) collected from Longyan drakes. Fifty settable eggs (egg weight > 63 g, with no soft shells, cracks, dirty, or double yolks) from each replicate were collected for 7 consecutive days from the second day after the first artificial insemination and stored at 17°C and 75% relative humidity (**RH**). All eggs were incubated in the same incubator (JXB2000; Dezhou Jingxiang Technology Co., Ltd, China) at 36.5 to 38.4°C and 45 to 65% RH from the 1st to 28th day of incubation. Eggs were turned (16 turns/d) throughout the incubation period and sprayed with water daily from the 15th day of incubation until they hatched. Nonfertile eggs were identified with a flashlight after incubation for 7 D. At the end of incubation, the healthy hatched ducklings were removed and recorded, and eggs that failed to hatch were counted. The hatchability of the fertile eggs was calculated. Healthy ducklings (clean and dry, free of deformities, and with bright eyes) were determined macroscopically as described by Tona et al. (2004).

Oviduct and Ovary-related Indices

The collected oviducts, ovaries, small yellow follicles (**SYF**; 3 mm < diameter < 8 mm), and large yellow follicles (**LYF**; diameter ≥ 8 mm) were dissected and weighed, and numbers of SYF and LYF were recorded. The weights of SYF and LYF were each expressed as proportions of ovarian weight.

Chemical Analysis of Plasma

The plasma concentrations of triglycerides, total cholesterol (**TCH**), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were determined spectrophotometrically in duplicates using kits (Jiancheng Bioengineering Institute, Nanjing, China; Chen et al., 2011).

Transcript Abundance of Lipid Metabolism-Related Genes

Total RNA was extracted from the frozen liver samples using an extraction kit (Invitrogen, Carlsbad, CA). All of the RNA samples were treated with DNAase (Takara, Biotechnology Co. Ltd, Dalian, China). After gel electrophoresis, their quality was confirmed based on the optical density at 260 and 280 nm ($1.7 < OD_{260:280} < 2.0$).

Complementary DNA (**cDNA**) was prepared by reverse transcription from 2.5 μg of high-quality RNA in a final volume of 25 μL according to the manufacturer's instructions (Promega, Madison, WI). The primers employed were designed based on GenBank sequences using Primer Premier 6.0 and prepared by Shanghai Shenggong Biological Company (Shanghai, China). The primer sequences of the examined genes are shown in Table 2.

Complementary DNA was amplified by PCR under optimal conditions, which consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 30 s at 59°C or 60°C, and 30 s at 72°C, with a final extension for 10 min at 72°C. Aliquots of PCR products were evaluated by electrophoresis on 1.5% agarose gel and the products excised from the gels were sequenced to verify their authenticity.

Table 2. Primer sequences used for quantitative real-time PCR.

Genes	Primer sequence (5′-3′)	Accession no.	Amplicon (bp)	Ta (°C)
ApoA-1	F: GCTGAGTACCAGGCCAAGGT R: GATGAAGCGGGTCTTGAGGT	XM 005009561.1	123	59
ApoVLDL-II	F: TGGTCAGTTCTTGGCGGATG R: TCACTGCTCATTGGGTCTCC	GQ180104.1	165	59
FAS	F: CAGCGGCAGTTGGTCAGTT R: GGCTCTCTCTCACATTGGCAG	AY613443.1	152	59
SREBP-1	F: ACCGCTCATCCATCAACGA R: GGCTGAGGTTCTCCTGCTTC	AY613441.1	156	59
ACC-α	F: GCTGCTGAAGAGGTTGGCTA R: AGATTGGAGAGCCTGGGACT	EF990143.1	142	59
CPT-1A	F: CCTGGTGGGCCACAAACTAT R: GAGCGGAACAGTTGATCCCA	XM_005022766.2	236	59
β-actin	F: GCTATGTCGCCCTGGATTT R: GGATGCCACAGGACTCCATAC	EF667345.1	174	60

Ta = annealing temperature; ApoA-1 = apolipoprotein A1; ApoVLDL-II = very low density apolipoprotein-II; FAS = fatty acid synthase; SREBP-1 = sterol regulatory element binding protein-1; ACC-α = acetyl CoA carboxylase α; *CPT-1A* = carnitine palmitoyltransferase-1A.

Quantitative real-time PCR was performed using an MXPro 3500 system (Stratagene, La Jolla, CA) with 1 μL of the cDNA product in a total volume of 20 μL, which contained 10 μL of SYBR-green PCR master Mix (Takara, Biotechnology Co. Ltd) and 0.5 μL (10 mM) of each primer. The specificity of the reaction was monitored by determining the product melting curve. The following protocol was used: denaturation for 30 s at 95°C, followed by 35 cycles of 20 s at 95°C, 30 s at 60°C, and 20 s at 72°C. The transcripts were quantified using a standard curve based on 10-fold serial dilutions of cDNA. Each sample was assayed in triplicate, and the standard deviations of the threshold cycle value did not exceed 0.5.

The relative abundance of the targeted genes was calculated using the ΔCt method ($R = 2^{-\Delta\Delta\ Ct}$), where R is the relative expression level of the target gene and ΔCt is the value obtained by subtracting the Ct value for β-actin mRNA from the Ct value for the target mRNA, as described previously (Ruan et al., 2015).

Statistical Analysis

Six replicates were used as the experimental units. The effects of ME and CP were analyzed by 2-way ANOVA using SAS (SAS Institute Inc., 2004). The statistical model used for analysis was as follows: $Y_{ijk} = \mu_{ijk} + E_i + P_j + (E \times P)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the replicate value of a given variable, μ_{ijk} is the mean, E_i is the effect of ME (i = 1, 2, or 3; 2,600, 2,500, or 2,400 kcal/kg), P_j is the effect of CP (j = 1, 2, or 3; 19, 18, or 17%), $(E \times P)_{ij}$ is the effect of the interaction between the 2 main effects, and ε_{ijk} is the random error. The data are expressed as means with SEM, derived from the ANOVA error mean square. Where the ANOVA indicated significant main effects or interactions, the corresponding means were compared using least significant difference tests. A quadratic regression equation based on 95% of the maximum or minimum response was used to estimate the dietary optimal ME to CP ratio for the corresponding means whenever an interaction was observed (Corzo et al., 2006). $P < 0.05$ indicated a significant difference.

RESULTS

Productivity Performance

As shown in Table 3, the ME and CP levels displayed significant interaction effects ($P < 0.05$) on egg production, egg mass, and feed conversion ratio. These measures were not affected by the increase of ME level at low CP, and negatively affected by the increase of ME at medium CP, while they were improved significantly with the increase of ME at high CP. According to the quadratic regression models, the optimal ME to CP ratios for egg production, egg mass, and feed conversion ratio were 12.9, 12.9, and 12.8 kcal/g protein, respectively.

BW and Abdominal Fat

The effects of dietary ME and CP on BW loss and abdominal fat in laying duck breeders are shown in Table 4. The abdominal fat in birds fed M_{ME} was 27% and 19% lower than in the birds fed H_{ME} and L_{ME} ($P < 0.05$), while the BW loss was not affected by the dietary ME or CP.

Incubation Indices

As shown in Table 5, the percentage of healthy ducklings resulting in the group fed H_{CP} was 13% and 11% higher than in those fed M_{CP} and L_{CP} ($P < 0.05$). There was a significant interaction ($P < 0.05$) between ME and CP levels in the hatchling weights, where the heaviest weights were obtained in $L_{ME}H_{CP}$, $M_{ME}M_{CP}$, $L_{ME}M_{CP}$, and $H_{ME}L_{CP}$, while the lightest weight was obtained in $L_{ME}L_{CP}$. There was no quadratic effect on

Table 3. Effects of dietary metabolizable energy (ME) and CP contents on the productivity performance of breeder laying ducks during the laying period (29 to 45 wk of age).

Variable[1]	ME:CP ratio (kcal/g)	ME intake (kcal/d)	CP intake (g/d)	Egg production[6] (%)	Egg weight (g)	Egg mass[6] (g/d)	Feed conversion ratio[5,6] (g/g)
Treatments[2] (n = 6)							
$H_{ME}H_{CP}$	13.8	419	30.4	83.9[a,b]	67.6	56.8[a,b]	2.85[b]
$M_{ME}H_{CP}$	13.2	402	30.4	77.7[a,b,c]	67.0	52.1[a,b,c]	3.12[a,b]
$L_{ME}H_{CP}$	12.7	386	30.4	78.1[a,b,c]	66.5	51.9[a,b,c]	3.15[a,b]
$H_{ME}M_{CP}$	14.4	419	29.0	74.9[b,c]	67.8	50.6[b,c]	3.19[a,b]
$M_{ME}M_{CP}$	13.9	402	29.0	84.5[a,b]	66.9	56.6[a,b]	2.86[b]
$L_{ME}M_{CP}$	13.3	386	29.0	85.7[a]	67.2	57.5[a]	2.81[b]
$H_{ME}L_{CP}$	15.3	419	27.4	72.6[c]	66.8	48.3[c]	3.37[a]
$M_{ME}L_{CP}$	14.7	402	27.4	70.2[c]	66.5	46.5[c]	3.50[a]
$L_{ME}L_{CP}$	14.1	386	27.4	71.6[c]	66.9	47.8[c]	3.39[a]
SEM[3]				2.25	0.30	1.37	0.08
Main effect of ME (n = 18)							
H_{ME}		419		77.1	67.4	51.9	3.14
M_{ME}		402		77.5	66.8	51.7	3.16
L_{ME}		386		78.5	66.9	52.4	3.12
SEM[3]				1.28	0.18	0.80	0.05
Main effect of CP (n = 18)							
H_{CP}			30.4	79.9[a]	67.0	53.6[a]	3.04[b]
M_{CP}			29.0	81.7[a]	67.3	54.9[a]	2.95[b]
L_{CP}			27.4	71.5[b]	66.7	47.5[b]	3.42[a]
SEM[3]				1.28	0.18	0.80	0.05
P-values[4]							
ME				0.80	0.23	0.88	0.88
CP				<0.01	0.32	<0.01	<0.01
ME × CP				<0.05	0.67	<0.01	<0.05

[1]H_{CP} = high CP (19%); M_{CP} = medium CP (18%); L_{CP} = low CP (17%); H_{ME} = high ME (2,600 kcal/kg); M_{ME} = medium ME (2,500 kcal/kg); L_{ME} = low ME (2,400 kcal/kg).
[2]Means separation in treatments shows significant interaction.
[3]Pooled SEM of treatments (n = 6 replicates with 12 birds per replicate); pooled SEM of main effect (n = 18 replicates with 12 birds per replicate).
[4]Means within a column and within an item lacking the same superscript are different ($P < 0.05$).
[5]All birds were given 161 g/d during the laying period from 29 to 45 wk of age.
[6]Regression equation based on dietary ME: CP ratio (kcal/g); quadratic equation: Y (egg production) = –1792 + 277 (ME: CP ratio)–10.2 (ME: CP ratio)^2, R^2 = 0.80, *P*-value = 0.040; Y (egg mass) = –1340 + 206 (ME: CP ratio)–7.60 (ME: CP ratio)^2, R^2 = 0.83, *P*-value = 0.030; Y (feed conversion ratio) = 90.4–12.9 (ME: CP ratio)–0.48 (ME: CP ratio)^2, R^2 = 0.85, *P*-value = 0.022; which yielded optimized ME: CP ratio value of 12.9, 12.9, and 12.8 kcal/g, respectively.

hatchling weights in response to the dietary ME to CP ratio.

Oviduct and Ovary-related Indices

The oviduct and ovary-related indices are summarized in Table 6. Of the examined indices, the relative weights of LYF and SYF were affected only by dietary CP ($P < 0.05$). The relative weight of the LYF/ovarian weight in the birds fed L_{CP} was lower than those of the birds fed H_{CP} and M_{CP} (0.67 vs. 0.77 and 0.79), while the SYF/ovarian weight in birds fed H_{CP} and L_{CP} were higher than in those fed M_{CP} ($P < 0.05$) (0.05 and 0.06 vs. 0.03).

Chemical Analyses of Plasma

With the exception of TCH, no differences were observed in the analyzed plasma components (Table 7). The plasma concentration of TCH in birds fed L_{ME} was higher ($P < 0.05$) than those in birds fed M_{ME} (4.70 vs.3.68 mmol/L).

Hepatic Expression of Genes Related to Lipid Metabolism

As shown in Table 8, relative hepatic expression of *ApoVLDL-II* was affected by CP, and the lowest expression was found in birds fed M_{CP} ($P < 0.05$). For *CPT-1A*, its relative expression was affected by both ME and CP levels per se, with a significant interaction between them ($P < 0.05$). Within the ME levels, the highest *CPT-1A* expression was obtained with the medium energy level, while the *CPT-1A* expression in the H_{CP} exceeded those obtained with the lower CP levels. The highest expression occurred with $M_{ME}H_{CP}$ and the lowest, representing about 43% that of $M_{ME}H_{CP}$, occurred with $L_{ME}M_{CP}$. No quadratic effect of *CPT-1A* relative hepatic expression in response to the dietary ME to CP ratio was observed.

DISCUSSION

Dietary ME has important roles in feed intake and nutrient metabolism, and subsequently in the growth

Table 4. Effects of dietary metabolizable energy (ME) and CP contents on the body weight (BW) loss and abdominal fat percentages of breeder laying ducks (29 to 45 wk of age).

Variable[1]	ME intake (kcal/d)	CP intake (g/d)	BW loss (g)	Abdominal fat (%)
Main effect of ME (n = 18)				
H_{ME}	419		43.4	0.79[a]
M_{ME}	402		43.7	0.58[b]
L_{ME}	386		44.0	0.72[a]
SEM[2]			20.6	0.05
Main effect of CP (n = 18)				
H_{CP}		30.4	34.5	0.71
M_{CP}		29.0	25.0	0.66
L_{CP}		27.4	71.7	0.72
SEM[2]			20.6	0.05
P-values[3]				
ME			0.99	<0.05
CP			0.25	0.68
ME × CP			0.63	0.82

[1]H_{CP} = high CP (19%); M_{CP} = medium CP (18%); L_{CP} = low CP (17%); H_{ME} = high ME (2,600 kcal/kg); M_{ME} = medium ME (2,500 kcal/kg); L_{ME} = low ME (2,400 kcal/kg).
[2]Pooled SEM of BW loss (n = 18 replicates with 12 birds per replicate); pooled SEM of abdominal fat (n = 18 replicates with 2 birds per replicate).
[3]Means within a column and within an item lacking the same superscript are different ($P < 0.05$).

Table 5. Effects of dietary metabolizable energy (ME) and CP contents on the incubation indices of breeder laying ducks (29 to 45 wk of age).

Variable[1]	ME:CP ratio	ME intake (kcal/d)	CP intake (g/d)	Fertility of set eggs (%)	Hatchability of fertile eggs (%)	Healthy ducklings (%)	Hatchling weight (g)
Treatments[2] (n = 6)							
$H_{ME}H_{CP}$	13.8	419	30.4	88.5	57.9	88.0	38.4[a,b]
$M_{ME}H_{CP}$	13.2	402	30.4	84.4	50.3	91.4	38.5[a,b]
$L_{ME}H_{CP}$	12.7	386	30.4	91.6	53.7	88.7	39.0[a]
$H_{ME}M_{CP}$	14.4	419	29.0	86.1	59.3	81.2	38.6[a,b]
$M_{ME}M_{CP}$	13.9	402	29.0	84.1	53.0	77.0	39.3[a]
$L_{ME}M_{CP}$	13.3	386	29.0	87.9	55.0	78.9	39.5[a]
$H_{ME}L_{CP}$	15.3	419	27.4	89.1	56.6	80.8	39.5[a]
$M_{ME}L_{CP}$	14.7	402	27.4	77.0	57.6	84.0	38.4[a,b]
$L_{ME}L_{CP}$	14.1	386	27.4	84.6	53.0	76.9	37.1[b]
SEM[3]				2.27	3.20	3.76	0.52
Main effect of ME (n = 18)							
H_{ME}		419		87.9	58.0	83.3	38.8
M_{ME}		402		86.8	53.6	84.1	38.7
L_{ME}		386		88.0	53.9	81.5	38.5
SEM[4]				1.31	1.79	2.17	0.32
Main effect of CP (n = 18)							
H_{CP}			30.4	88.2	54.0	89.4[a]	38.6
M_{CP}			29.0	86.0	55.8	79.0[b]	39.1
L_{CP}			27.4	83.6	55.7	80.6[b]	38.4
SEM[4]				1.31	1.79	2.17	0.32
P-values[5]							
ME				0.48	0.17	0.68	0.81
CP				0.22	0.72	<0.05	0.22
ME × CP				0.32	0.63	0.73	<0.05

[1]H_{CP} = high CP (19%); M_{CP} = medium CP (18%); L_{CP} = low CP (17%); H_{ME} = high ME (2,600 kcal/kg); M_{ME} = medium ME (2,500 kcal/kg); L_{ME} = low ME (2,400 kcal/kg).
[2]Means separation in treatments shows significant interaction.
[3]Pooled SEM (n = 6 replicates with 50 eggs per replicate).
[4]Pooled SEM (n = 18 replicates with 50 eggs per replicate).
[5]Means within a column and within an item lacking the same superscript are different ($P < 0.05$).

and reproductive performance of laying birds (Alvarenga et al., 2013). Offering more than 512 kcal ME/bird/d, from the diet with 2,700 kcal/kg, was reported to increase abdominal fat deposition and negatively affect the carcass quality in Pekin duck (Fan et al., 2008). Murugesan and Persia (2013) found that the reduction in energy intake from 2,880 to 2,790 kcal/kg diet in Hy-Line W36 laying hens did not change the energy partitioned toward production or maintenance, but reduced the fat pad. In the cur-

Table 6. Effects of dietary metabolizable energy (ME) and CP contents on the reproductive organ indices of breeder laying ducks at the end of the study (45 wk of age).

Variable[1]	ME intake (kcal/d)	CP intake (g/d)	Oviduct weight (g)	Ovarian weight (g)	LYF number	SYF number	LYF weight/ovarian weight	SYF weight/ovarian weight
Main effect of ME (n = 18)								
H_{ME}	419		32.0	42.5	4.83	12.8	0.74	0.05
M_{ME}	402		36.2	44.9	4.36	15.2	0.70	0.05
L_{ME}	386		33.9	48.6	5.03	15.4	0.78	0.05
SEM[2]			1.34	3.42	0.24	1.41	0.03	0.01
Main effect of CP (n = 18)								
H_{CP}		30.4	34.4	47.3	4.86	16.4	0.77^{a}	0.05^{a}
M_{CP}		29.0	35.3	47	4.85	11.8	0.79^{a}	0.03^{b}
L_{CP}		27.4	32.5	41.8	4.52	15.3	0.67^{b}	0.06^{a}
SEM[2]			1.34	3.42	0.24	1.41	0.03	0.01
P-values[3]								
ME			0.10	0.45	0.14	0.37	0.14	0.86
CP			0.44	0.32	0.52	0.06	<0.05	<0.05
ME × CP			0.25	0.92	0.17	0.94	0.13	0.76

[1]H_{CP} = high CP (19%); M_{CP} = medium CP (18%); L_{CP} = low CP (17%); H_{ME} = high ME (2,600 kcal/kg); M_{ME} = medium ME (2,500 kcal/kg); L_{ME} = low ME (2,400 kcal/kg); LYF = large yellow follicles, diameter > 8 mm; SYF = small yellow follicles, diameter = 3–8 mm.
[2]Pooled SEM (n = 18 replicates with 2 birds per replicate).
[3]Means within a column and within an item lacking the same superscript are different ($P < 0.05$).

Table 7. Effects of dietary metabolizable energy (ME) and CP contents on the plasma chemical variables of breeder laying ducks at the end of the study (45 wk of age).

Variable[1]	ME intake (kcal/d)	CP intake (g/d)	TG (mmol/L)	TCH (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Main effect of ME (n = 18)						
H_{ME}	419		5.15	$3.90^{a,b}$	2.33	2.11
M_{ME}	402		5.55	3.68^{b}	2.02	2.34
L_{ME}	386		5.94	4.70^{a}	3.09	2.51
SEM[2]			0.80	0.30	0.34	0.28
Main effect of CP (n = 18)						
H_{CP}		30.4	4.87	4.03	2.69	2.28
M_{CP}		29.0	6.63	3.72	2.33	2.06
L_{CP}		27.4	5.14	4.54	2.42	2.63
SEM[2]			0.80	0.30	0.34	0.28
P-values[3]						
ME			0.79	<0.05	0.08	0.61
CP			0.26	0.16	0.74	0.36
ME × CP			0.45	0.90	0.36	0.82

[1]H_{CP} = high CP (19%); M_{CP} = medium CP (18%); L_{CP} = low CP (17%); H_{ME} = high ME (2,600 kcal/kg); M_{ME} = medium ME (2,500 kcal/kg); L_{ME} = low ME (2,400 kcal/kg); TG = triglycerides, TCH = total cholesterol, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol.
[2]Pooled SEM (n = 18 replicates with 2 birds per replicate).
[3]Means within a column and within an item lacking the same superscript are different ($P < 0.05$).

rent study, dietary ME displayed a significant effect on the abdominal fat percentage in laying duck breeders, where the lowest level was found in birds fed 2,500 kcal of ME/kg diet (M_{ME}), which provided 402 kcal/bird/d ME intake. Dietary ME, however, did not affect the reproductive performance of the breeding ducks in the present study, which is consistent with previous findings in laying hens (Novak et al., 2008). In laying Isa-brown and White Leghorn hens, the egg production was not affected by increasing the dietary ME level from 2,680 to 2,810 kcal/kg to provide the same energy intake, or from 2,645 to 2,976 kcal/kg for increasing energy intake to 16.7 kcal/d (Peguri and Coon, 1991; Grobas et al., 1999). These studies indicate that increasing the energy intake by changing the diet composition mainly lead to increased body fat deposition rather than affecting the egg production. The lipid metabolism-related variables in plasma of laying duck breeders and their hepatic gene expression showed that dietary ME had significant effects on plasma TCH concentration and expression of the key fatty acid metabolic enzyme *CPT-1A*, which is a crucial enzyme in fatty acid catabolism via the transport of fatty acids into mitochondria and by controlling the flux of fatty acids that enter the β-oxidation pathway. The lowest plasma TCH concentration and highest *CPT-1A* expression were obtained with 2,500 kcal/kg (M_{ME}), which could contribute in explaining the low abdominal fat content with this diet.

Dietary proteins are particularly important for growth, immune function, and production of hormones

Table 8. Effects of dietary metabolizable energy (ME) and CP contents on hepatic expression of genes related to lipid metabolism of breeder laying ducks at the end of the study (45 wk of age).

Variable[1]	ME:CP ratio	ME intake (kcal/d)	CP intake (g/d)	*ApoA-1*	*ApoVLDL*-II	*FAS*	*SREBP-1*	*ACC-α*	*CPT-1A*
Treatments[2] (n = 6)									
$H_{ME}H_{CP}$	13.8	419	30.4	1.06	1.22	1.07	0.99	1.03	1.04[b]
$M_{ME}H_{CP}$	13.2	402	30.4	1.20	1.27	1.16	1.18	1.12	1.87[a]
$L_{ME}H_{CP}$	12.7	386	30.4	1.25	0.76	1.13	1.16	1.13	1.25[b]
$H_{ME}M_{CP}$	14.4	419	29.0	1.12	0.69	1.22	1.04	1.06	1.05[b]
$M_{ME}M_{CP}$	13.9	402	29.0	1.09	0.77	1.02	1.07	1.13	1.30[b]
$L_{ME}M_{CP}$	13.3	386	29.0	1.01	0.54	1.28	1.19	0.83	0.80[b]
$H_{ME}L_{CP}$	15.3	419	27.4	1.15	1.04	1.09	1.01	1.07	1.16[b]
$M_{ME}L_{CP}$	14.7	402	27.4	0.98	1.21	1.13	0.82	1.07	1.15[b]
$L_{ME}L_{CP}$	14.1	386	27.4	1.19	1.02	1.09	0.95	1.27	1.21[b]
SEM[3]				0.15	0.17	0.14	0.10	0.10	0.16
Main effect of ME (n = 18)									
H_{ME}		419		1.11	0.98	1.13	1.01	1.05	1.08[b]
M_{ME}		402		1.09	1.08	1.10	1.02	1.10	1.44[a]
L_{ME}		386		1.15	0.77	1.17	1.10	1.08	1.08[b]
SEM[4]				0.09	0.09	0.08	0.06	0.06	0.09
Main effect of CP (n = 18)									
H_{CP}			30.4	1.17	1.08[a]	1.12	1.11	1.09	1.39[a]
M_{CP}			29.0	1.07	0.66[b]	1.17	1.10	1.00	1.05[b]
L_{CP}			27.4	1.11	1.09[a]	1.11	0.93	1.13	1.17[a,b]
SEM[4]				0.09	0.09	0.08	0.06	0.06	0.09
P-values[5]									
ME				0.89	0.06	0.86	0.53	0.82	<0.01
CP				0.74	<0.01	0.84	0.06	0.29	<0.05
ME × CP				0.74	0.70	0.80	0.45	0.12	<0.05

[1] H_{CP} = high CP (19%); M_{CP} = medium CP (18%); L_{CP} = low CP (17%); H_{ME} = high ME (2,600 kcal/kg); M_{ME} = medium ME (2,500 kcal/kg); L_{ME} = low ME (2,400 kcal/kg); *ApoA-1* = apolipoprotein A1, *ApoVLDL-II* = very low density apolipoprotein-II, *FAS* = fatty acid synthase, *SREBP-1* = sterol regulatory element binding protein-1, *ACC-α* = acetyl CoA carboxylase α, *CPT-1A* = carnitine palmitoyltransferase-1A.
[2] Means separation in treatments shows significant interaction.
[3] Pooled SEM (n = 6 replicates with 2 birds per replicate).
[4] Pooled SEM (n = 18 replicates with 2 birds per replicate).
[5] Means within a column and within an item lacking the same superscript are different ($P < 0.05$).

and enzymes (Patterson and Burley, 2017). The importance of dietary proteins for the egg laying birds has been also demonstrated in some other species, e.g., the red-billed quelea (Jones and Ward, 1976), lesser snow goose (Ankeny and MacInnes, 1978), and lesser black-backed gull (Houston et al., 1983). Novak et al. (2008), however, showed that decreasing the dietary CP from 16 to 13% reduced egg weight, egg mass, amount of excreted nitrogen, and impaired the feed conversion ratio in laying hens aged from 18 to 60 wk. Minimizing the dietary CP, without affecting the normal performance, can decrease the feed costs and reduce the nitrogen excretion, which therefore lessen the consequent ammonia emission from poultry manure. In growing turkey, Pack et al. (1996) demonstrated that amino acid supplementation to the diets containing CP levels at 90% of NRC (1994) recommendation reduced N excretion by 16.4% without adversely affecting growth performance. These results indicate that optimizing the diet of hens with a lower CP content could have a positive impact on the environment without adverse effects on their productive performance. In the present study, it was found that changing the dietary CP levels did not affect the BW loss of laying duck breeders, but clutch formation was influenced. Increasing the dietary CP from 17 to 18% and 19% in the laying duck breeders showed heavier LYF relative weight and even heavier SYF relative weight with 19% CP. This is probably because the effect of dietary CP on the BW is not so pronounced after completion of the skeletal and somatic growth in adult birds. During the breeding period, the recovery of protein from the losses of female BW can be associated with clutch formation, which further masks the decline in body fat (Mawhinney et al., 1999). Therefore, the laying ducks breeders in the current study fed diets containing 18% and 19% CP were in better condition compared to those fed 17% CP, where they produced more eggs with better feed conversion efficiency. Because of the high formation of yellow follicles, the hepatic expression of the gene for the yolk precursor *ApoVLDL-II* and the key fatty acid metabolic enzyme *CPT1A* were significantly higher in the diets of higher CP levels. Considering the improvements in egg production, formation of yellow follicles, and the hepatic expression of *ApoVLDL-II* and *CPT1A*, as well as the highest proportion of healthy ducklings, the results of this study indicate that 19% CP was beneficial for maintaining longer laying peak period in the breeding ducks and for improving hatchling outcome.

Moraes et al. (2014) suggested that an optimal maternal dietary energy to protein ratio during the rearing phase of broiler breeders can maximize the BW of female progeny. Spratt and Leeson (1987) demonstrated that the weight of chicks responded significantly to the change in energy to protein ratio, and the highest chick weight was found in breeders fed a diet containing 18.1 kcal ME/g protein. In the current study, dietary ME and CP also interacted with respect to hatchling weight; however, the average egg weight was not affected, and the best hatchling weights were obtained with 13.3 and 15.3 kcal ME/g protein. Pearson and Herron (1982) showed that the dietary ME and CP had significant interactions on egg production in laying hens aged from 21 to 36 wk, and the highest production was obtained with 16.6 kcal ME/g protein. Spratt and Leeson (1987) suggested that 385 kcal ME and 19 g protein (20.3 kcal ME/g protein) was sufficient to maintain normal production performance throughout the peak egg production period of broiler breeder hens. In the current study, the diet with ME to CP ratio ranging from 12.8 to 12.9 kcal/g protein optimized egg production, egg mass, and feed conversion in laying duck breeders. These values are less than NRC (1994) recommendations of 19.3 kcal/g protein for laying Pekin ducks and Leghorn laying hens. The results of the present study with laying duck breeders and those with layer and broiler chicken breeders discussed above suggest that the optimal dietary energy to protein ratio required to maximize productivity is variable; it may differ with the experimental diets, species and breed, and/or age of birds.

In conclusion, disregarding the CP level, the diet with 2,500 kcal/kg of ME increased lipid metabolism in the liver and decreased abdominal fat; meanwhile, 19% CP improved reproductive performance and outcome irrespective of ME level. The diet with ME of 12.9 kcal/g protein optimized both egg production and egg mass, while the feed conversion was optimized with the diet containing 12.8 kcal ME/kg. Using 2,451 kcal ME/kg at 19% dietary CP, i.e., ME to CP ratio of 12.9 kcal/g protein, maximized the reproductive performance and hatchling outcome of Longyan laying duck breeders.

ACKNOWLEDGMENTS

This work was supported by the Earmarked Fund for China Agriculture Research System (Grant No. CARS-42–13), the National Key Research and Development Program (2018YFD0501504), the Science and Technology Program for Pearl River Nova of Guangzhou City (Grant No. 201710010159), Key Project of the Science and Technology Program of Guangzhou City (201804020091), Operating Funds for Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition (Grant No. 2014B030301054). W. Bruce Currie (Emeritus Professor, Cornell University) made suggestions on presentation.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

REFERENCES

Alvarenga, R. R., P. B. Rodrigues, M. G. Zangeronimo, L. Makiyama, E. C. Oliveira, R. T. Freitas, R. R. Lima, and V. M. Bernardino. 1970. Validation of prediction equations to estimate the energy values of feedstuffs for broilers: performance and carcass yield. Asian-Australas J. Anim. Sci. 26:1474–1483.

Ankney, C. D., and C. D. MacInnes. 1978. Nutrient reserves and reproductive performance of female Lesser Snow Geese. The Auk. 95:459–471.

Bu, Z., P. Xie, S. Y. Fu, H. B. Tong, and X. Dai. 2015. Effect of energy and protein levels on performance, egg quality, and nutrient digestibility of laying pigeons. J. Appl. Poult. Res. 24:371–379.

Chen, J., M. Wang, Y. Kong, H. Ma, and S. Zou. 2011. Comparison of the novel compounds creatine and pyruvateon lipid and protein metabolism in broiler chickens. Animal. 5:1082–1089.

Corzo, A., W. A. Dozier, 3rd, and M. T. Kidd. 2006. Dietary lysine needs of late-developing heavy broilers. Poult. Sci. 85:457–461.

European Economic Community 1989. Hen eggs and egg products. Council Directive 89/437/EEC. Off. J. L. 212:22.07.

Fan, H. P., M. Xie, W. W. Wang, S. S. Hou, and W. Huang. 2008. Effects of dietary energy on growth performance and carcass quality of white growing pekin ducks from two to six weeks of age. Poult. Sci. 87:1162–1164.

Grobas, S., J. Mendez, C. De Blas, and G. G. Mateos. 1999. Laying hen productivity as affected by energy, supplemental fat, and linoleic acid concentration of the diet. Poult. Sci. 78:1542–1551.

Houston, D., P. Jones, and R. Sinly. 1983. The effect of female body condition on egg laying in Lesser black-backed gulls Larus fuscus. J. Zool. 200:509–520.

Jones, P., and P. Ward. 1976. The level of reserve protein as the proximate factor controlling the timing of breeding and clutch-size in the red-billed Quelea Quelea quelea. Ibis. 118:547–574.

Li, F., L. M. Zhang, X. H. Wu, C. Y. Li, X. J. Yang, Y. Dong, A. Lemme, J. C. Han, and J. H. Yao. 2013. Effects of metabolizable energy and balanced protein on egg production, quality, and components of Lohmann Brown laying hens. J. Appl. Poult. Res. 22:36–46.

Lin, R. L., H. P. Chen, R. Rouvier, and J. P. Poivey. 2014. Selection and crossbreeding in relation to plumage color inheritance in three chinese egg type duck breeds (Anas platyrhynchos). Asian Australas. J. Anim. Sci. 27:1069–1074.

Mawhinney, K., A. W. Diamond, and F. P. Kehoe. 1999. The use of energy, fat, and protein reserves by breeding Great Black-backed Gulls. Can. J. Zool. 77:1459–1464.

Ministry of Agriculture of China. 2004. Feeding Standard of Chicken. Standards Press of China, Beijing, China.

Ministry of Agriculture, Forestry and Fishery (MAFF), Japan. 1992. Japanese Feeding Standards—Poultry. Chuo Chikusan Sha, Tokyo, Japan.

Moraes, T. G., A. Pishnamazi, E. T. Wenger, Mba II, R. A. Renema, and M. J. Zuidhof. 2014. Effect of maternal dietary energy and protein on live performance and yield dynamics of broiler progeny from young breeders. Poult. Sci. 93:2818–2826.

Murugesan, G. R., and M. E. Persia. 2013. Validation of the effects of small differences in dietary metabolizable energy and feed restriction in first-cycle laying hens. Poult. Sci. 92:1238–1243.

Novak, C. L., H. M. Yakout, and J. Remus. 2008. Response to varying dietary energy and protein with or without enzyme supplementation on Leghorn performance and economics. 2. Laying period. J. Appl. Poult. Res. 17:17–33.

NRC 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Oke, U. K., U. Herhert, and A. H. Akinmutimi. 2003. Early lay characteristics and haematology of pearl guinea fowls as influenced by dietary protein and energy levels. Int. J. Poult. Sci. 2:128–132.

Pack, W., P. R. Ferket, J. Thomas, and L. N. Grimes. 1996. Carcass yield and N balance of turkey toms fed high and low crude protein diets supplemented with menefee humate. Poult. Sci. 75(Suppl.1): 116(Abstr).

Patterson, P. H., and H. K. Burley. 2017. Chapter 29 - Modifying Protein in Feed A2 - Hester, Pages 309–317 in Egg Innovations and Strategies for Improvements. Y Patricia, ed. Acad. Press, San Diego.

Pearson, R. A., and K. M. Herron. 1982. Effects of maternal energy and protein intakes on the incidence of malformations and malpositions of the embryo and time of death during incubation. Br. Poult. Sci. 23:71–77.

Peguri, A., and C. Coon. 1991. Effect of temperature and dietary energy on layer performance. Poult. Sci. 70:126–138.

Ruan, D., Y. Lin, W. Chen, S. Wang, W. Xia, A. Fouad, and C. Zheng. 2015. Effects of rice bran on performance, egg quality, oxidative status, yolk fatty acid composition, and fatty acid metabolism-related gene expression in laying ducks. Poult. Sci. 94:2944–2951.

SAS Institute Inc 2004. SAS User's Guide: Statistics. Version 9.1. SAS Institue Inc., Gary, NC.

Spratt, R. S., and S. Leeson. 1987. Broiler breeder performance in response to diet protein and energy. Poult. Sci. 66:683–693.

Tarasewicz, Z., D. Szczerbińska, M. Ligocki, M. Wiercińska, D. Majewska, and K. Romaniszyn. 2006. The effect of differentiated dietary protein level on the performance of breeder quails. Anim. Sci. Pap. Rep. 24:207–216.

Thongwittaya, N., and I. Tasaki. 1992. Energy and protein requirements of Khaki Campbell x Thai Native laying ducks. Asian Australas. J. Anim. Sci. 5:365–368.

Tona, K., O. Onagbesan, B. De Ketelaere, E. Decuypere, and V. Bruggeman. 2004. Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and chick posthatch growth to forty-two days. J. Appl. Poult. Res. 13:10–18.

Xia, W., A. M. Fouad, W. Chen, D. Ruan, S. Wang, Q. Fan, Y. Wang, Y. Cui, and C. Zheng. 2017. Estimation of dietary arginine requirements for Longyan laying ducks. Poult. Sci. 96:144–150.

Xia, W. G., H. X. Zhang, Y. C. Lin, and C. T. Zheng. 2015. Evaluation of dietary calcium requirements for laying Longyan shelducks. Poult. Sci. 94:2932–2937.