Effects of dietary lysine on productivity, reproductive performance, protein and lipid metabolism-related gene expression in laying duck breeders

D. Ruan,[*,†] A. M. Fouad,[*,‡] Y. N. Zhang,[*] S. Wang,[*] W. Chen,[*] W. G. Xia,[*] S. Q. Jiang,[*] L. Yang,[†] and C. T. Zheng[*,1]

[*]*Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Key Laboratory of Animal Nutrition and Feed Science (South China) of Ministry of Agriculture; State Key Laboratory of Livestock and Poultry Breeding; Guangdong Pubic Laboratory of Animal Breeding and Nutrition; Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, P. R. China;* [†]*College of Animal Science, South China Agricultural University, Guangzhou 510640, P. R. China; and* [‡]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt*

ABSTRACT This study investigated whether dietary lysine (Lys) affects productive performance and expression of genes related to protein and lipid metabolism in laying duck breeders. *Longyan* duck breeders (n = 540, 19 wk of age) were randomly assigned to 6 groups with 6 replicates of 15 birds each. Breeders were fed diets with 6 total Lys levels (6.4, 7.2, 8.0, 8.8, 9.6, and 10.4 g/kg) for 26 wk duration. Egg production, egg weight, egg mass, feed conversion ratio, hatchability, hatchling weight, albumen weight, eggshell weight, yolk weight, and yolk proportion increased with dietary Lys levels ($P < 0.05$). Dietary Lys level had a linear ($P < 0.05$) and quadratic ($P < 0.05$) effects on maternal hepatic expression of mechanistic target of rapamycin, eukaryotic translation initiation factor 4E binding protein 1, ubiquitin conjugating enzyme E2K (*UBE2K*), cathepsin B (*CTSB*), and quadratically ($P < 0.05$) increased the concentrations of plasma Lys, leucine, threonine, and tryptophan in duck breeders. In contrast, maternal dietary Lys suppressed expression of proteasome 26S subunit, *UBE2K*, and *CTSB* in the liver of hatchlings. Moreover, relative expression of peroxisome proliferator-activated receptors alpha, carnitine palmitoyltransferase 1A, and very low density apolipoprotein-II increased linearly ($P < 0.05$) and quadratically ($P < 0.05$), and that of VLDL receptor (*VLDLR*) decreased quadratically ($P < 0.05$) in the liver of duck breeders with increasing dietary Lys levels; hepatic triglyceride and cholesterol contents were reduced. Maternal dietary Lys suppressed hepatic expression of *VLDLR* in the hatchlings. A diet containing 8.6 g Lys/kg promoted protein turnover and lipid metabolism in laying duck breeders, which positively reflected in the productivity and reproductive performance.

Key words: lysine, proteolysis, lipid metabolism, performance, laying duck breeders

2019 Poultry Science 0:1–12
http://dx.doi.org/10.3382/ps/pez361

INTRODUCTION

Lysine (**Lys**) is the second limiting amino acid in plant-based poultry feeds and it is used as the reference amino acid to establish the ideal amino acid ratios (Fernandez et al., 1994; Baker, 1997). Like other essential amino acids, Lys mainly participates in protein metabolism (Tesseraud et al., 1996; Edwards et al., 1999; Urdaneta-Rincon and Leeson, 2004). In avian species, Lys deficiency reduces the growth and laying performance, muscle growth, egg mass (Prochaska et al., 1996; Kakhki et al., 2016; Fouad et al., 2018; Meloche et al., 2018), protein deposition (Tesseraud et al., 1996; Edwards et al., 1999; Urdaneta-Rincon and Leeson, 2004), and decreases FCR, while increasing fat deposition (Grisoni et al., 1991). In addition, maternal Lys intake affected fertility, hatchability, and performance of progeny, but excess levels of Lys were detrimental to offspring performance (Mejia et al., 2012, 2013; Ciacciariello and Tyler, 2013). The mechanistic target of rapamycin (**MTOR**), one of the major amino acid-induced signaling pathways, is involved in protein synthesis (Deng et al., 2014). The proteolytic system involves lysosomal, ubiquitin-proteasome-dependent, and Ca-dependent systems. Although Lys can stimulate protein synthesis and suppress proteolysis has been demonstrated in chickens (Tesseraud et al., 1996, 2009), but so far, no comparable published report on Lys in protein metabolism exists for duck breeders, especially concerning protein synthesis and degradation in their progeny.

Received January 29, 2019.
Accepted June 9, 2019.
[1]Corresponding author: zhengcht@163.com

Dietary Lys is the exogenous precursor for L-carnitine biosynthesis and is involved in lipid metabolism. Dietary Lys supplementation enhances the levels of carnitine, and affects the activities of coenzymes related to lipid metabolism, as well as promoting fatty acid β-oxidation (Hevia and Visek, 1980; Rabie and Szilágyi, 1998). Several studies have reported that growing chicks fed low protein diets deficient in Lys exhibit liver lipid infiltration and high cholesterol and promote abdominal fat deposition (Schmeisser et al., 1983). Breeder hens given dietary L-carnitine decrease carcass fat and increase breast meat in the progeny (Kidd et al., 2005). Little is known about this type of lipid infiltration, although it is possible that inadequate protein synthesis in the deficiency state may cause fundamental damage to the enzyme systems related to lipid metabolism and simultaneously lead to an increased content of lipids in liver.

In spite of the physiological importance of Lys and its relation with product quality, data regarding Lys requirements of duck breeders were not covered in NRC (NRC, 1994) and remains to be estimated. The *Longyan* duck breeders are the most popular laying ducks for duckling production in southern China. Also, egg production leads to changes in lipid and protein metabolism by duck breeders to cover the needs of yolk and albumen formation (Braun et al., 2001). Therefore, the main goals of the present work sought to investigate the impact of Lys in protein and lipid metabolism in laying duck breeders; it also examined whether maternal dietary Lys promoted protein and lipid metabolism in their offspring.

MATERIALS AND METHODS

Experimental Design, Diets, and Bird Husbandry

All experimental procedures were approved by the Animal Care and Use Committee of the Institute of Animal Science, Guangdong Academy of Agriculture Sciences and performed in accordance with animal welfare and ethics. The trial used a randomized complete block design with 6 dietary treatments. The treatments consisted of a non-supplemented basal diet (control) and that diet supplemented with 5 graded concentrations of Lys. Peanut meal was used to gain a low Lys treatment, and L-Lys HCl (98.5% purity, CJ CheilJedang Co., Ltd, Shanghai, China) to the basal diet at the expense of a carrier to obtain experimental diets. Each treatment contains 6.4, 7.2, 8.0, 8.8, 9.6, and 10.4 g/kg L-Lys, respectively. All nutrients were kept at the same levels to satisfy the nutritional requirements for *Longyan* laying duck breeders except the L-Lys content of the basal diet (Table 1). The contents of Lys and other amino acids in each diet were analyzed using a Hitachi L-8900 Amino Acid Analyzer (Hitachi High Technologies Corporation, Tokyo, Japan). The analyzed levels of dietary Lys in basal and experimental diets were 6.2, 7.1, 8.1, 9.0, 9.8, and 10.6 g/kg of diet, respectively. Male *Longyan* duck breeders were fed a standard commercial diet containing 10.87 MJ/kg metabolizable energy/kg, 170 g/kg crude protein, 8.0 g/kg Ca, 3.8 g/kg available phosphorus, 9.5 g/kg Lys, and 4.5 g/kg Met.

A total of 540 19-wk-old female *Longyan* laying duck breeders (Longyan Duck Breeding Corporation, Fujian, China) with the same genetic background in terms of the parental generation and with comparable BW, were randomly allocated to 1 of 6 treatments, with 6 replicates (cages) of 15 birds for each treatment balanced for BW. The ducks were individually housed in galvanized steel cages (length 42 cm × width 30 cm × height 50 cm; Guangzhou Huanan Poultry Equipment Co., Ltd., Guangzhou, China). All duck breeders were handled in accordance with the *Longyan* duck breeder management guidelines for lighting, feeding, and allowed ad libitum access to tap water. The ambient temperature varied from 12 to 30°C with the seasons during the 26-wk experimental period. The 82.5 g pelleted feed was introduced twice daily at 07:00 and 15:00 h. A 16 h light-8 h dark photoperiod was applied throughout the study. At 43 wk of age, each breeder was artificially inseminated twice weekly with 100 μL of pooled semen. A total of 1,800 eggs (50 eggs from each replicate) were collected over 5 sequential days between 43 and 44 wk from the second day after the first artificial insemination, the eggs were weighed, labeled, and stored in a dark controlled-temperature room (18°C and 75 to 80% relative humidity), and then incubated in the incubator (JXB2000, Dezhou Jingxiang Technology Co., Ltd, Shandong, China); followed the procedure as described in a companion study from our laboratory (Ruan et al., 2018). The eggs were candled on day 6 and day 18 to eliminate infertile eggs and dead embryos. The fertility and hatchability were determined along with hatchling weights of ducklings being recorded on the hatching day.

Sample Collections and Preparations

At the end of the experiment (at 45 wk of age), 12 breeders per treatment were selected at random (excluding obvious outliers in BW, 2 from each replicate) for sampling. Heparinized blood was collected from the wing vein, centrifuged at 2,000 × g for 15 min at 4°C, then the plasma was collected and stored at −20°C until subsequent analysis. The birds were then stunned and exsanguinated. Samples were collected from the liver, which were rinsed rapidly with ice-cold PBS (pH 7.4), snap-frozen in liquid N_2, and stored at −80°C for further analysis. After hatching, 12 ducklings from each treatment group (2 from each replicate) were stunned, exsanguinated, and samples of the liver were harvested and processed, as above.

Table 1. Composition and nutrient content of the basal diet for laying duck breeders (as-fed basis).

Item	Content, g/kg	Nutrient composition[2]	Level, g/kg
Corn	541.0	Metabolisable energy, MJ/kg	10.46
Wheat bran	120.0	CP	172.5
Soyabean meal	105.0	Ca	36.0
Peanut meal	120.0	Total P	5.6
DL-Methionine (99%)	2.4	Available P	3.5
L-Threonine hydrochloride (98.5%)	0.6	Total Lys	6.2
L-Isoleucine (98.5%)	1.3	Total Met	4.5
L-Tryptophan (98%)	0.2	Total Met+Cys	7.2
Limestone	85.0	Total Thr	6.0
Dicalcium phosphate	11.5	Total Trp	2.1
Sodium chloride	3.0	Total Ile	6.6
Premix[1]	10.0		
Total	1000.0		

[1]The premix provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2,640 IU; vitamin E, 20 mg; vitamin K_3, 2.75 mg; vitamin B_1, 4.4 mg; vitamin B_2, 9.0 mg; vitamin B_6, 4.4 mg; vitamin B_{12}, 0.02 mg; choline, 500 mg; nicotinic acid, 30 mg; pantothenic acid, 22 mg; folic acid, 1.1 mg; biotin, 0.22 mg; Fe, 60 mg; Cu, 8.0 mg; Mn, 100 mg; Zn, 90 mg; I, 0.5 mg; Se, 0.2 mg; Co, 0.26 mg.

[2]Total Lys, Met, Met+Cys, Thr, Ile, and CP were measured values in the mixed feed. Each value is based on triplicate determinations. Other nutrient compositions are calculated values.

Productive and Reproductive Performance Determination

The feed intake was recorded daily on a per replicate basis. The numbers of total, soft, broken, and shell-less eggs were recorded daily by replicate. The eggs produced were individually weighed and graded daily. Egg production (%) (100 × number of laid eggs per day/number of breeders), egg weight (g), egg mass (g/d), average daily feed intake, and FCR (g feed/g egg) were calculated daily, per replicate. Data were pooled before analysis. Mortality was recorded daily in order to calculate mortality rate and to adjust FCR. The fertility and hatchability were also calculated per replicate.

Egg Quality Assessment

On the last day of the experiment, a total of 24 normal eggs per treatment were randomly collected, excluding any eggs that were excessively large or small, rough, misshapen, or cracked to determine the quality characteristics. Yolk color score, albumen height were assessed and Haugh unit was calculated with an EA-01 Egg Multi-Tester (ORKA Food Technology, Ramat HaSharon, Israel). Shell static compression strength was determined by an EFR-01 Egg Force analyzer (ORKA Food Technology, Ramat HaSharon, Israel). Shell thickness, including membranes, was measured at the equator, and blunt and pointed ends using an electronic micrometer. Both egg length (long axis) and width at half length (short axis) were measured with a digital caliper and shape index was determined as width × 100/length. The eggs were weighed, and yolks were separated using an egg separator, rolled on a moist paper towel to remove adhering albumen, weighed, and expressed as percentages of egg weight. The eggshells with shell membranes were rinsed with distilled water, weighed after drying at 105°C, and expressed as percentages of egg weight. Finally, the albumen weight was calculated by subtracting the yolk and shell weight from the total egg weight, and expressed as percentages of egg weight.

Biochemical Assay of Plasma and Tissue Samples

The plasma contents of total protein, albumin, uric acid, high density lipoprotein cholesterol (**HDL-C**), low density lipoprotein cholesterol (**LDL-C**), triglyceride (**TG**), and total cholesterol (**TCH**) were measured in duplicate based on a spectrophotometric method using an automatic biochemistry analyzer (Roche e702, Roche Diagnostics, Mannheim, Germany). The plasma content of very low density lipoprotein cholesterol (V**LDL-C**) was determined in duplicate with an enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Approximately 0.2 g of liver tissues was pulverized in liquid N_2 and was extracted in 3.0 mL of chloroform:methanol (2:1, v/v) mixture according to the method of Folch et al. (1957). The hepatic concentrations of TG and TCH in breeders and ducklings were determined in duplicate with kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Plasma Free Amino Acid Analysis

Frozen heparinized plasma samples (0.4 mL) were first thawed at 4°C and then deproteinized by mixing with 3 volumes of salicylic acid (10%, wt/vol) in a 2.0 mL micro-centrifuge tube. The solution was vortexed and then centrifuged at 12,000 × *g* for 15 min at 4°C. The supernatant was collected and then passed through a filter (0.2 μm) before analysis. Amino acids were determined by ion-exchange chromatography with

Table 2. Primers of target genes used for quantitative real-time PCR.

Transcript[1]	Accession number	Primer sequence (5′-3′)	Size (bp)
MTOR	XM_0,050,21280.3	F: GGAATGAACCGTGATGACCG R: AGCATTTGACTGAGAGGGCT	228
EIF2B1	XM_0,050,16325.3	F: CCAAGCTCTGTCATCCGTTC R: GACCAGGTCCACTTTCTCCA	245
EIF4EBP1	XM_0,050,30133.2	F: TTGAGAACAACCACGTGCAG R: TGTTCTCAGCAGGGAAGGAG	216
RPS6KA1	XM_0,050,17482.3	F: TAATCGTGCTGTGGACTGGT R: AGCCTGAACTTCTCCAGCAT	235
UBE2K	XM_02,127,9433.1	F: AAGACGCAGTAGTGGCAAAC R: AATAACAGATGGTGATGCTCCT	295
PSMD1	XM_01,310,4440.2	F: GCTGAGTACCAGGCCAAGGT R: GATGAAGCGGGTCTTGAGGT	155
CTSB	XM_02,127,7964.1	F: TGCTTGGAGGTATCTGGCAA R: TACAAGGGAGAGCCTGCTTC	245
FAS	AY613443.1	F: CAGCGGCAGTTGGTCAGTT R: GGCTCTCTCTCACATTGGCAG	152
PPARα	NM_0,013,10383.1	F: CAGAGTCATCCTTGCAGG R: GTCAAGATTGGAGAAGCC	229
SREBP1	AY613441.1	F: ACCGCTCATCCATCAACGA R: GGCTGAGGTTCTCCTGCTTC	156
ApoVLDL-II	GQ180104.1	F: TGGTCAGTTCTTGGCGGATG R: TCACTGCTCATTGGGTCTCC	165
VLDLR	JF950612.1	F: GCCATATTCCAGCCACAACT R: CTATTGCCATTGCCCCCACTA	159
HMGCR	XM_0,050,11606.3	F: GGGAGCTTGCTGTGAGAATG R: CTCGAGTCATCCCATCTGCT	203
CPT1A	XM_0,050,22766.3	F: CCTGGTGGGCCACAAACTAT R: GAGCGGAACAGTTGATCCCA	236
β-actin	EF667345.1	F: GCTATGTCGCCCTGGATTT R: GGATGCCACAGGACTCCATAC	174

[1]*MTOR* = mechanistic target of rapamycin; *EIF2B1* = eukaryotic translation initiation factor 2B subunit alpha; *EIF4EBP1* = eukaryotic translation initiation factor 4E binding protein 1; *RPS6KA1* = ribosomal protein S6 kinase A1; *UBE2K* = ubiquitin conjugating enzyme E2K; *PSMD1* = proteasome 26S subunit, non-ATPase 1; *CTSB* = cathepsin B; *FAS* = fatty acid synthase; *PPARα* = peroxisome proliferator-activated receptors alpha; *SREBP1*, sterol regulatory element binding protein 1; *ApoVLDL-II* = very low density apolipoprotein-II; *VLDLR* = very low density lipoprotein receptor; *HMGCR* = 3-hydroxy-3-methylglutaryl-CoA reductase; *CPT1A* = carnitine palmitoyltransferase 1A.

post-column ninhydrin derivatization using a Hitachi L-8900 Amino Acid Analyzer (Hitachi High Technologies Corporation, Tokyo, Japan), and were quantified on the basis of authentic standards (Sigma Chemicals, St. Louis, MO) using EZChrom Elite version 3.1.5b Software.

RNA Isolation, Reverse Transcription, and Real-Time PCR

Total RNA was isolated from the frozen liver using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction, and dissolved in RNase-free water. The quantity and purity of total RNA were qualified by a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, Pittsburgh, PA) and the integrity of the RNA was verified by denaturing polyacrylamide gel electrophoresis. Only samples that had an OD260/280 > 1.8, OD260/230 > 2.0, and a RNA integrity number > 7.0 were used for further sequencing.

In brief, a mass of 1.0 μg of total RNA from each sample was used to obtain cDNA by reverse transcription using a PrimeScript RT regent kit with gDNA Eraser (TaKaRa, Tokyo, Japan). RT-qPCRs were performed on a CFX 96 real-time PCR detection system (Bio-Rad, Hercules, CA) using iTaq Universal SYBER Green Supermix (TaKaRa, Tokyo, Japan). Primers for the genes of interest were designed with Primer Premier 5.0 based on duck sequences in GenBank, as shown in Table 2; they were obtained from Shanghai ShengGong Biological Company. PCR procedures, run in triplicate, consisted of a pre-denaturation at 95°C for 30 s, followed by 39 cycles of 95°C for 5 s, and annealing and amplification for 30 s. The $2^{-\Delta\Delta CT}$ method was used to analyze the relative mRNA expression of each target gene (Livak and Schmittgen, 2001). The expression of β-actin was selected as an internal control to normalize the expression of the targeted genes. Data are shown with further normalization to values obtained from the basal diet.

Statistical Analysis

Replicate ($n = 6$) served as the experimental unit, where 2 breeders and ducklings were sampled per replicate unless stated otherwise. A complete randomized design with 6 Lys treatments was applied. Effects of dietary Lys were analyzed by one-way Analysis of Variance using the general linear model procedure in SAS (SAS Institute Inc., Cary, NC). Differences among means were assessed using Duncan's multiple range tests at $P < 0.05$ probability levels. Results

Table 3. Effects of the dietary lysine level on the productivity and reproductive performance of duck breeders in the peak laying period from 19 to 45 wk of age.[1]

	Dietary Lys level, g/kg							*P*-value[4]		
Indices[2]	6.4	7.2	8.0	8.8	9.6	10.4	SEM[3]	Lys	Linear	Quadratic
Laying performance										
Egg production, %	75.91^{b}	80.13^{a,b}	82.74^{a}	83.93^{a}	85.52^{a}	84.38^{a}	1.551	0.002	<0.001	0.036
Egg weight, g	63.50^{b}	65.74^{a}	66.40^{a}	66.44^{a}	66.76^{a}	66.09^{a}	0.423	0.009	0.004	0.008
Egg mass, g/d	48.24^{c}	52.70^{b}	54.96^{a,b}	55.52^{a,b}	57.06^{a}	55.77^{a,b}	0.896	<0.001	<0.001	0.012
FCR[5], g feed/g egg	3.46^{a}	3.24^{a,b}	3.06^{b,c}	3.03^{b,c}	2.93^{c}	3.01^{b,c}	0.058	<0.001	<0.001	<0.001
Abdominal fat, %	1.10^{a}	1.05^{a}	0.95^{a,b}	0.79^{b}	0.56^{c}	0.55^{c}	0.053	0.032	0.025	0.043
Broken egg rate, %	0.34	0.42	0.33	0.33	0.38	0.33	0.068	0.409		
Misshapen egg rate, %	0.09	0.06	0.10	0.18	0.09	0.11	0.037	0.348		
Reproductive performance										
Fertility, %	93.05	92.28	91.60	94.96	94.58	95.98	1.604	0.189		
Hatchability, %	75.27^{b}	77.15^{b}	80.05^{a,b}	83.90^{a,b}	87.31^{a}	81.78^{a,b}	2.751	0.026	0.006	0.312
BW at 1-day of age, g	33.47^{b}	33.69^{b}	34.38^{b}	36.19^{a,b}	38.00^{a}	36.07^{a,b}	0.442	0.038	0.015	0.428

[1]Each value represents the mean of 6 replicates (14 birds per replicate).
[2]FCR = feed conversion ratio.
[3]Stand error of mean (n = 6).
[4]Lys = treatment effect; when significant, linear and quadratic effects were tested.
[5]All birds were given 160 g/day during the peak-laying period.

Table 4. Effects of the dietary lysine level on the egg composition and quality of duck breeders during the laying period from 19 to 45 wk of age.[1]

	Dietary Lys level, g/kg							*P*-value[4]		
Indices[2]	6.4	7.2	8.0	8.8	9.6	10.4	SEM[3]	Lys	Linear	Quadratic
Egg composition										
Egg weight (g)	60.39^{c}	61.32^{b,c}	63.28^{a,b}	64.97^{a}	65.47^{a}	65.07^{a}	0.727	<0.001	<0.001	0.075
Yolk weight (g)	20.37c	21.06^{b,c}	21.52^{b,c}	23.04^{a}	22.85^{a}	21.80^{a,b}	0.400	<0.001	<0.001	0.002
TG (mg/g)	273.1	269.3	260.0	267.3	269.2	259.4	4.275	0.634		
TCH (mg/g)	27.0	26.8	26.5	26.2	26.0	26.4	0.480	0.774		
Albumen weight (g)	34.24^{b}	34.36^{b}	36.01^{a,b}	36.20^{a,b}	36.60^{a}	37.05^{a}	0.446	0.004	<0.001	0.420
Eggshell weight (g)	5.80^{c}	5.84^{c}	5.98^{b,c}	6.16^{a,b}	6.17^{a,b}	6.22^{a}	0.092	0.026	<0.001	0.338
Yolk proportion (%)	33.68^{b}	34.42^{a,b}	34.17^{a,b}	35.59^{a}	34.88^{a,b}	33.81^{b}	0.469	0.041	0.651	0.009
Albumen proportion (%)	56.74	56.06	56.49	55.02	55.91	56.43	0.480	0.108		
Eggshell proportion (%)	9.59	9.52	9.34	9.51	9.22	9.55	0.099	0.136		
Egg quality										
Eggshell thickness (mm)	0.456	0.485	0.479	0.484	0.475	0.491	0.009	0.884		
Eggshell strength (N)	39.81	40.57	41.45	40.64	40.64	40.67	1.235	0.511		
Egg shape index	75.05	74.50	74.67	74.75	74.09	74.12	0.422	0.475		
Haugh unit	74.25	75.73	76.13	78.04	76.45	76.81	1.768	0.891		
Yolk color	5.75	5.79	5.83	5.83	5.79	5.69	0.185	0.859		

[1]Each value represents the mean of 6 replicates (4 eggs per replicate).
[2]TG = triglyceride; TCH = total cholesterol.
[3]Stand error of mean (n = 6).
[4]Lys = treatment effect; when significant, linear and quadratic effects were tested.

are presented as means with their standard errors of the mean. Only when the main effect was significant ($P < 0.05$), orthogonal comparisons were applied for linear and quadratic effects of Lys supplementations. Quadratic models ($Y = c + bX + aX^2$) were fitted to the responses of the dependent variables to dietary Lys content. The dietary concentration of Lys at which the response reached 95% of the maximum was used to estimate the requirement (Dozier et al., 2009).

RESULTS

Productive and Reproductive Performance

As shown in Table 3, hatchability, and 1 D duckling weight increased in a linear manner ($P < 0.001$) with increasing dietary Lys. Egg production, egg weight, and egg mass increased, and FCR and abdominal fat decreased, in a linear and quadratic manner ($P < 0.05$) as dietary Lys concentrations increased. The maximal effects were obtained with 9.6 g/kg Lys. There were no significant effects ($P > 0.05$) of dietary Lys levels on fertility.

Egg quality

Among the egg quality indices (Table 4), the albumen and eggshell weight increased in a linear ($P < 0.05$) manner with dietary Lys concentrations, whereas the yolk weight and yolk proportion increased, and then decreased quadratically ($P < 0.01$) with dietary Lys; maximal values were obtained with 8.8 g/kg. There

Table 5. Effects of the dietary lysine level on biochemical indices for duck breeders at 45 wk of age.[1]

Indices[2]	Dietary Lys level, g/kg						SEM[3]	*P*-value[4]		
	6.4	7.2	8.0	8.8	9.6	10.4		Lys	Linear	Quadratic
TP (g/L)	43.5^{b}	48.9^{a,b}	50.1^{a}	52.8^{a}	53.5^{a}	50.6^{a}	5.012	0.030	0.070	0.003
ALB (g/L)	13.2	15.0	14.9	15.6	15.9	14.6	1.588	0.425		
UA (μmol/L)	438	386	375	363	370	400	80.5	0.645		
HDL-C (mmol/L)	0.04^{b}	0.05^{a,b}	0.08^{a,b}	0.10^{a}	0.09^{a,b}	0.06^{a,b}	0.012	0.006	0.047	0.009
LDL-C (mmol/L)	0.23	0.23	0.30	0.31	0.27	0.31	0.106	0.741		
VLDL-C (mmol/L)	0.46^{a}	0.44^{a}	0.39^{a,b}	0.31^{b}	0.37^{a,b}	0.37^{a,b}	0.025	0.008	<0.001	0.022
TCH (mmol/L)	3.76^{a}	3.64^{a}	3.24^{a,b}	2.52^{b}	2.56^{b}	3.43^{a,b}	0.325	0.040	0.046	0.032
TG (mmol/L)	8.82	8.97	9.33	8.13	8.31	8.82	0.958	0.459		

[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]TP = total protein; ALB = albumin; UA = uric acid; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; VLDL-C = very low density lipoprotein cholesterol; TG = total triglyceride; TCH = total cholesterol.
[3]Stand error of mean (n = 6).
[4]Lys = treatment effect; when significant, linear and quadratic effects were tested.

Table 6. Effects of the dietary lysine level on plasma concentrations of free amino acids in laying duck breeders at 45 wk of age.[1]

Indices	Dietary Lys level, g/kg						SEM[2]	*P*-value[3]		
	6.4	7.2	8.0	8.8	9.6	10.4		Lys	Linear	Quadratic
Nutritionally indispensable amino acids (μg/mL)										
Arginine	39.9^{b}	40.9^{a,b}	41.5^{a,b}	42.2^{a}	39.7^{b}	39.0^{c}	1.423	0.090	0.048	0.018
Cysteine	12.0	10.4	10.6	13.0	12.3	12.2	1·138	0.753		
Histidine	13.3	14.3	14.5	14.3	15.1	13.6	0.879	0.737		
Isoleucine	13.9^{b}	14.1^{b}	15.8^{b}	15.7^{b}	18.3^{a}	16.2^{a,b}	0.827	0.008	0.002	0.319
Leucine	23.7^{c}	24.7^{b,c}	27.1$^{a–c}$	27.7^{a,b}	29.9^{a}	28.3^{a}	0.874	0.014	<0.001	0.034
Lysine	37.5^{b}	42.8^{a,b}	44.9^{a,b}	47.5^{a,b}	54.2^{a}	45.5^{a,b}	0.998	0.013	0.025	0.028
Methionine	17.8	18.0	16.2	18.6	16.4	17.8	0.969	0.903		
Phenylalanine	23.0	22.8	23.7	25.3	24.3	24.9	0.821	0.906		
Threonine	23.1^{b}	26.8^{a,b}	30.4^{a,b}	34.8^{a}	30.9^{a,b}	28.7^{a,b}	0.981	0.033	0.045	0.018
Tryptophan	33.0^{c}	34.0^{c}	36.6^{b,c}	39.8^{b}	45.4^{a}	38.7^{b}	0.911	0.020	0.032	0.025
Tyrosine	31.9	33.8	30.8	35.6	34.0	30.7	2.111	0.530		
Valine	27.0	26.8	27.5	30.3	34.1	30.8	0.886	0.071		
Nutritionally dispensable amino acids (μg/mL)										
Alanine	71.8	73.5	84.1	99.3	85.6	85.5	2.682	0.033	0.142	0.184
Aspartate	6.9	7.0	7.2	8.3	9.9	7.7	0.962	0.275		
Glutamate	11.0^{b}	11.4^{b}	13.8^{a,b}	13.9^{a,b}	17.4^{a}	15.4^{a,b}	1.335	0.038	0.010	0.462
Glycine	26.2^{b}	29.3^{a,b}	29.9^{a,b}	30.4^{a,b}	33.9^{a}	30.4^{a,b}	2.115	0.026	0.012	0.591
Proline	25.5	25.4	25.8	27.6	27.6	27.5	1.402	0.887		
Serine	67.6^{c}	78.5^{b,c}	83.1$^{a–c}$	91.1^{a,b}	98.8^{a}	91.9^{a,b}	2.925	0.032	<0.001	0.212

[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]Stand error of mean (n = 6).
[3]Lys = treatment effect; when significant, linear and quadratic effects were tested.

were no effects ($P > 0.05$) of increasing Lys on the eggshell thickness, egg shape index, eggshell strength, yolk color, albumen, and eggshell proportion.

Biochemical Analyses

As shown in Table 5, the plasma contents of total protein, HDL-C, VLDL-C, and TCH responded in a linear and quadratic ($P < 0.05$) manner as dietary Lys concentrations increased; maximal quadratic effects occurred with 8.8 or 9.6 g/kg Lys. There were no significant effects ($P > 0.05$) of increasing Lys on plasma contents of LDL-C, TG, albumin, and uric acid.

Plasma-Free Amino Acid Profile

The amino acid composition in plasma is provided in Table 6. The proportion of leucine, Lys, threonine, and tryptophan increased in a linear and quadratic manner ($P < 0.05$) as dietary Lys concentrations increased; maxima occurred with 9.6 g/kg Lys, except 8.8 g/kg for threonine. The proportions of isoleucine, glutamate, glycine, and serine increased linearly ($P < 0.05$) with dietary Lys concentrations.

Liver Cholesterol and Triglyceride Content in Laying Duck Breeders and their Progeny

Table 7 shows that the hepatic content of TCH and TG in laying duck breeders decreased quadratically ($P < 0.05$) as the dietary Lys content increased; minima occurred with 8.8 or 9.6 mg/kg Lys. Simultaneously, there was a linear decrease ($P < 0.05$) in hepatic content of TG in newly hatched ducklings as Lys content of the breeders' diet increased, but there was no effect ($P > 0.05$) of maternal dietary Lys of duck

Table 7. Effects of the dietary lysine level on liver cholesterol and triglyceride content in laying duck breeders and hatchlings.[1]

	Dietary Lys level, g/kg							*P*-value[4]		
Indices[2]	6.4	7.2	8.0	8.8	9.6	10.4	SEM[3]	Lys	Linear	Quadratic
Duck breeders										
TG (mg/g)	38.5[a]	36.5[a,b]	35.3[a,b]	31.8[b]	33.4[b]	37.6[a]	4.012	0.032	0.155	0.018
TCH (mg/g)	240.5[a]	225.5[a]	220.6[a,b]	182.6[b]	190.3[b]	222.4[a,b]	45.5	0.045	0.289	0.036
Hatchlings										
TG(mg/g)	29.9[a]	28.8[a]	27.3[a,b]	23.6[b]	21.0[c]	25.4[b]	4.012	0.025	0.012	0.212
TCH(mg/g)	240.5	246.2	215.5	202.6	214.6	222.4	32.8	0.345		

[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]TCH = total cholesterol; TG = total triglyceride.
[3]Stand error of mean (n = 6).
[4]Lys = treatment effect; when significant, linear and quadratic effects were tested.

Table 8. Effects of the dietary lysine level on relative hepatic expression of genes related to protein and lipid metabolism in laying duck breeders at 45 wk of age.[1]

	Dietary Lys level, g/kg							*P*-value[4]		
Indices[2]	6.4	7.2	8.0	8.8	9.6	10.4	SEM[3]	Lys	Linear	Quadratic
Protein metabolism										
MTOR	1.03[b]	1.12[a,b]	1.34[a,b]	1.63[a]	1.46[a,b]	1.33[a,b]	0.123	0.019	0.012	0.028
EIF2B1	1.01	1.14	1.02	1.16	1.28	0.95	0.166	0.758		
EIF4EBP1	1.00[b]	1.23[a,b]	1.61[a]	1.79[a]	1.69[a]	1.23[a,b]	0.163	0.012	0.062	0.002
RPS6KA1	1.05	1.16	1.18	1.37	1.13	1.38	0.145	0.244		
UBE2K	1.00[b]	1.27[a,b]	1.44[a,b]	1.84[a]	1.69[a]	1.42[a,b]	0.097	0.034	0.007	0.008
PSMD1	1.03	1.04	1.18	1.17	1.39	1.28	0.113	0.438		
CTSB	1.01[c]	1.21[b,c]	1.75[a–c]	2.39[a,b]	2.44[a]	2.06[a,b]	0.352	0.037	0.003	0.159
Lipid metabolism										
FAS	1.00	1.13	0.96	0.97	1.08	0.98	0.146	0.694		
SREBP1	1.02	0.81	0.95	1.13	0.88	0.91	0.135	0.469		
PPARα	1.00[b]	0.96[b]	1.43[a,b]	2.08[a]	1.91[a]	1.65[a,b]	0.110	0.008	0.016	0.026
CPT1A	1.00[b]	1.05[b]	1.03[b]	1.66[a]	1.46[a,b]	1·24[b]	0.097	0.016	0.023	0.040
ApoVLDL-II	1.00[c]	1.19[c]	1.89[a,b]	2.88[a]	2.34[a]	1·67[b]	0.157	0.045	0.027	<0.001
VLDLR	1.03[a]	1.11[a]	1.05[a]	0.50[b]	0.33[b]	0·34[b]	0.101	<0.001	<0.001	<0.001
HMGCR	1.02	0.93	1.18	1.05	1.18	1·05	0.135	0.542		

[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]*MTOR* = mechanistic target of rapamycin; *EIF2B1* = eukaryotic translation initiation factor 2B subunit alpha; *EIF4EBP1* = eukaryotic translation initiation factor 4E binding protein 1; *RPS6KA1* = ribosomal protein S6 kinase A1; *UBE2K* = ubiquitin conjugating enzyme E2K; *PSMD1* = proteasome 26S subunit, non-ATPase 1; *CTSB* = cathepsin B; *FAS* = fatty acid synthase; *PPARα* = peroxisome proliferator-activated receptors alpha; *SREBP1*, sterol regulatory element binding protein 1; *ApoVLDL-II* = very low density apolipoprotein-II; *VLDLR* = very low density lipoprotein receptor; *HMGCR* = 3-hydroxy-3-methylglutaryl-CoA reductase; *CPT1A* = carnitine palmitoyltransferase 1A.
[3]Stand error of mean (n = 6).
[4]Lys = treatment effect; when significant, linear and quadratic effects were tested.

breeders on TCH contents in liver of newly hatched ducklings.

Expression of Genes Related to Protein and Lipid Metabolism in the Liver of Laying Duck Breeders

As shown in Table 8, dietary Lys levels increased the hepatic abundance of *MTOR*, ubiquitin conjugating enzyme E2K (***UBE2K***), cathepsin B (***CTSB***), proliferator-activated receptor alpha (***PPARα***), and carnitine palmitoyltransferase 1A (***CPT1A***) transcripts in a linear ($P < 0.05$) and quadratic ($P < 0.05$) manner in laying duck breeders. The relative expression of eukaryotic translation initiation factor 4E binding protein 1(***EIF4EBP1***) increased quadratically ($P < 0.01$), whereas very low density apolipoprotein II (***ApoVLDL-II***) transcripts increased and those of VLDL receptor (***VLDLR***) decresed linearly and quadratically ($P < 0.05$) with dietary Lys concentrations. With the exception of *CTSB* (9.6 g/kg), maximal changes occurred with 8.8 g/kg Lys.

Expression of Genes Related to Protein and Lipid Metabolism in the Liver of Hatchlings

Maternal Lys linearly ($P < 0.001$) decreased the hepatic expression of *EIF4EBP1*, *UBE2K*, *PSMD1*, *CTSB*, *FAS,* and *VLDLR* in newly hatched ducklings (Table 9). There was a linear ($P < 0.05$) and quadratic ($P < 0.05$) effect of maternal dietary Lys on the hepatic expression of ribosomal protein S6 kinase 1 (***RPS6KA1***), 3-hydroxy-3-methylglutaryl-CoA reductase (***HMGCR***), and *CPT1A* in the hatchlings; these showed maximal changes with 9.6 g/kg maternal dietary Lys. There were no significant effects ($P > 0.05$) on the hepatic

Table 9. Effects of the maternal dietary lysine level of duck breeders at 45 wk of age on relative hepatic expression of genes related to protein and lipid metabolism in hatchlings.[1]

	Dietary Lys level, g/kg							*P*-value[4]		
Indices[2]	6.4	7.2	8.0	8.8	9.6	10.4	SEM[3]	Lys	Linear	Quadratic
Protein metabolism										
MTOR	1.01	0.88	0.86	0.98	0.92	0.89	0.107	0.451		
EIF2B1	1.01	1.14	1.02	1.22	0.95	1.14	0.152	0.282		
EIF4EBP1	1.02^{a,b}	1.25^{a}	0.98^{a,b}	0.85^{a,b}	0.72^{a,b}	0.52^{b}	0.115	0.019	0.001	0.217
RPS6KA1	1.01^{a}	1.06^{a}	0.67^{b}	0.37^{b}	0.31^{b}	0.42^{b}	0.095	<0.001	<0.001	0.039
UBE2K	1.02^{a}	0.84^{a,b}	0.75^{a,b}	0.72^{b}	0.67^{b}	0.63^{b}	0.077	0.019	<0.001	0.232
PSMD1	1.03^{a}	0.97^{a}	0.92^{a,b}	0.61^{b,c}	0.51^{c}	0.60^{b,c}	0.105	0.003	<0.001	0.517
CTSB	1.01^{a}	0.97^{a,b}	0.72^{b,c}	0.69^{b,c}	0.45^{c}	0.44^{c}	0.254	<0.001	<0.001	0.388
Lipid metabolism										
FAS	1.03^{a}	0.97^{a}	0.95^{a}	0.65^{a,b}	0.41^{b}	0.62^{b}	0.111	0.011	<0.001	0.563
SREBP1	1.00	1.01	1.19	1.01	1.03	0.90	0.129	0.968		
PPARα	1.01	0.96	0.93	0.88	0.91	0.89	0.113	0.663		
CPT1A	1.03^{a}	0.84^{a,b}	0.62^{b}	0.48^{b}	0.45^{b}	0.74^{a,b}	0.106	0.027	0.015	0.012
ApoVLDL-II[†]	—	—	—	—	—	—	—	—		
VLDLR	1.00^{a}	1.10^{a}	1.05^{a}	0.76^{b,c}	0.54^{c}	0.86^{a,b}	0.084	<0.001	<0.001	0.256
HMGCR	1.01^{a}	0.99^{a}	0.83^{a,b}	0.40^{c}	0.36^{c}	0.59^{b,c}	0.077	<0.001	<0.001	0.031

[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]*MTOR* = mechanistic target of rapamycin; *EIF2B1* = eukaryotic translation initiation factor 2B subunit alpha; *EIF4EBP1* = eukaryotic translation initiation factor 4E binding protein 1; *RPS6KA1* = ribosomal protein S6 kinase A1; *UBE2K* = ubiquitin conjugating enzyme E2K; *PSMD1* = proteasome 26S subunit, non-ATPase 1; *CTSB* = cathepsin B; *FAS* = fatty acid synthase; *PPARα* = peroxisome proliferator-activated receptors alpha; *SREBP1*, sterol regulatory element binding protein 1; *ApoVLDL-II* = very low density apolipoprotein-II; *VLDLR* = very low density lipoprotein receptor; *HMGCR* = 3-hydroxy-3-methylglutaryl-CoA reductase; *CPT1A* = carnitine palmitoyltransferase 1A.
[3]Stand error of mean (n = 6).
[4]Lys = treatment effect; when significant, linear and quadratic effects were tested.
[†]Not detected.

Table 10. Estimations of the dietary lysine requirements based on non-linear regressions of egg weight, albumen weight, egg mass, and FCR on dietary Lys concentrations.

Dependent variables	Regression equation*	R^2	*P*	Dietary Lys requirement, g/kg[†]
Egg production	$Y = 5.010 + 16.605X–0.901X^2$	0.688	0.036	8.8
Egg weight	$Y = 30.484 + 8.037X–0.444X^2$	0.878	0.008	8.6
Egg mass	$Y = –21.883 + 16.636X–0.881X^2$	0.904	0.012	9.0
Feed conversion ratio	$Y = 7.510–0.953X + 0.049X^2$	0.762	0.001	9.1
Hatchability	$Y = –6.250 + 18.797X–0.977X^2$	0.702	0.031	9.1

*Y is the dependent variable and X the dietary Lys concentration, g/kg.
[†]Dietary Lys requirement = the optimal dietary Lys concentration according to each regression equation, g/kg.

expression of *MTOR*, *SREBP1*, and *PPARα* in the hatchlings, and *ApoVLDL-II* transcripts were not detected.

Estimates of the Dietary Lys Requirements of Laying Duck Breeders

Dietary Lys requirements of laying duck breeders as estimated by quadratic regression analyses are shown in Table 10. The dietary Lys requirements for *Longyan* duck breeders from 19 to 48 wk of age for optimizing egg production, egg weight, egg mass, FCR, and hatchability were 8.8, 8.6, 9.0, 9.1, and 9.1 g/kg, respectively.

DISCUSSION

In the present study, dietary Lys content had positive effects on the egg production, egg weight, egg mass, FCR, hatchability, and weight of hatchlings, but did not affect the breakages, abnormalities, and fertility. In previous studies using Cobb 500 broiler breeders, Mejia et al. (2012, 2013) found improvements in egg production, egg weight, egg mass, fertility, hatchability, and hatchling weight after increasing the digestible Lys level from 4.9 to 9.8 g/kg, while fertility and hatchability with 5.0 exceeded that with 7.0 g/kg. In the same strain of commercial *Longyan* laying ducks used here (22 to 38 wk of age), there was no improvement in egg production, egg weight, egg mass, or FCR after increasing Lys concentrations from 7.5 to 9.5 g/kg (Fouad et al., 2018). In contrast, increasing Lys concentrations from 6.5 to 9.5 g/kg for 8 wk during the peak laying period in *Linwu* laying ducks significantly improved egg mass and FCR without affecting egg production and egg weight (Huang et al., 2016), while in Hy-line laying hens aged 32 to 44 wk, improvements in the egg production, egg weight, egg mass, and FCR were obtained by increasing the level of Lys from 6.6 to 8.1 g/kg (Kakhki et al., 2016). In quail, increasing dietary Lys level from 8.8 to 11.8 g/kg optimized egg production, egg mass, and FCR (Lima et al., 2016), whereas in American White

laying pigeons, egg production significantly increased, egg weight significantly decreased by increasing Lys levels (Chang et al., 2018). Therefore, previous studies using different birds, strains and/or Lys concentrations in basal diet varied in responses to increasing dietary Lys level. However, retarded reproductive organs and liver as well as impaired protein and lipid biosynthesis were reported in birds that consumed diets containing less Lys than their requirements, which explained the reduced laying performance (Hiramoto et al., 1990; Tesseraud et al., 1996; Tian et al., 2019). As the prevalent laying breed in southern China, optimizing nutrient supply for *Longyan* duck breeders is of economic importance, so the present findings are commercially relevant.

The present study demonstrated that yolk weight, albumen weight, eggshell weight, and yolk proportion increased significantly in response to supplemental dietary Lys; other indices of egg quality were unchanged. Our previous study of *Longyan* laying ducks showed that eggshell weight, eggshell proportion, and eggshell thickness decreased linearly by increasing dietary Lys from 7.5 to 9.5 g/kg but the egg shape index, Haugh unit, yolk color, yolk weight, yolk proportion, albumen weight, and albumen proportion did not change (Fouad et al., 2018), whereas the egg composition and quality of *Linwu* laying ducks were unchanged by increasing Lys level in their diets (Huang et al., 2016). Similarly, in laying hens, there was no improvement in Haugh unit, yolk color, egg composition, or eggshell quality when different Lys levels added to the basal diets (Souza et al., 2014; Kakhki et al., 2016; Kumair et al., 2016), while Chang et al. (2018) observed a significant improvement in yolk color and a significant decline in eggshell thickness without affecting Haugh unit and egg composition in laying pigeons from increasing dietary Lys content. In avian species, dietary Lys supplementation enhances calcium and phosphorus utilization as well as improving protein bio-synthesis and lipid metabolism in liver (Tesseraud et al., 1996; Chang et al., 2018; Tian et al., 2019). These changes may explain why the albumen weight, yolk weight, and eggshell weight were enhanced, thereby increasing the egg and hatchling weight.

Amino acids are known to be anabolic factors that affect protein metabolism. It is well known that amino acids can regulate gene expression at both transcriptional and translational level via the MTOR signal pathway. MTOR regulates mRNA translation by phosphorylating its effectors: EIF4EBP1 and RPS6K1 (Corradetti and Guan, 2006). The major proteolytic pathway is the ATP-dependent ubiquitin-proteasome system, which is also ubiquitous throughout the body and degrades ubiquitin-conjugated proteins via the 26S proteasome including a proteolytic core known as 20S proteasome (Lecker et al., 2006), while the major protease of lysosomal proteolysis, CTSB, also contributes to protein degradation (Bechet et al., 2005). The present study provides evidence that relative expression of genes related to both protein synthesis and proteolysis was increased in liver of duck breeders by dietary Lys supplementation. In contrast, increased maternal Lys downregulated the hepatic expression of *UBE2K*, *PSMD1*, and *CTSB* in hatchlings. Previous research has shown that the ubiquitin-proteasome pathway is upregulated during the transition to sexual maturity in broiler breeders, suggesting a critical role of skeletal muscle as a source of amino acids for egg formation (Ekmay et al., 2013). Ekmay et al. (2014) reported that age and production rate rather than dietary energy and protein levels appeared to be the main drivers of Lys partitioning in broiler breeder hens. In growing chickens, Lys deficiency reduced muscle and liver protein deposition by activating lysosomal, Ca^{2+}-dependent, and ubiquitin-proteasome-dependent proteolytic systems (Tesseraud et al., 1996, 2009). Similar findings have been reported in muscles of broiler chickens where proteolysis declined by adding methionine to the deficient diet as a result of down-regulating expression of cathepsin L2 and Atrogin1 (Del Vesco et al., 2015). Ciacciariello and Tyler (2013) revealed that there was strong relationship between increasing levels of maternal Lys intake with overall production of hatchlings from hens at 38 wk of age to 21 D, but excess levels of Lys were detrimental to offspring performance. The proportion of essential amino acid (Lys, leucine, threonine, and tryptophan) increased in plasma of duck breeders in the present study as dietary Lys concentrations increased, which better explained the increased protein turnover in the liver of breeders and the increased albumen weight. This study appears to provide the first evidence in any avian species of suppressed expression of genes related to proteolysis in hatchling liver due to increasing maternal dietary Lys. The effect on progeny, therefore, was consequential when devising the correct nutritional strategy for breeders.

The liver is the primary site of fatty acid and cholesterol synthesis in poultry. Earlier studies showed that birds fed diets deficient in Lys activated *de novo* lipogenesis and increased body fat deposition, but supplementing Lys modulated lipogenesis and reduced body fat accumulation (Schmeisser et al., 1983; Sibbald and Wolynetz, 1986; Moran and Bilgili, 1990 Carew et al., 2005). Schmeisser et al. (1983) reported that excess Lys stimulated cholesterol biosynthesis in chicks. In laying hens, Lys levels had no effect on plasma TG, TCH, HDL-C, and HDL-C concentrations (Kakhki et al., 2016). In laying pigeons, increasing dietary Lys levels reduced serum TCH but not TG (Chang et al., 2018). In the study presented here, decreased abdominal fat percentage, plasma VLDL, TCH, liver TG, and TCH concentrations of duck breeders resulted from supplementing Lys in the diet (>8.8 g/kg). SREBP1 is an important transcription factor regulating hepatic fatty acid and cholesterol metabolism (Kim et al., 2002). PPARα as ligand-activated nuclear receptors participated in the transcriptional regulation of lipid metabolism and involved in hepatic fatty acid oxidation (Kersten, 2008). Carnitine palmitoyltransferase is a key enzyme for fatty acid catabolism via the transport

of fatty acids into mitochondria and by controlling the flux of fatty acids entering the β-oxidation pathway (McGarry and Brown, 1997). In rats with dietary Lys deficiency, there was impaired synthesis of the lipoprotein complex for transportation of hepatic lipid, mitochondrial transport, and oxidation of fatty acids (Tanphaichitr, et al., 1976). Lys, as the exogenous precursor for L-carnitine biosynthesis, is clearly involved in lipid metabolism. L-carnitine elevates *PPARα* expression in hepatocytes and plays an important part in the protective effect, which might contribute to the amelioration of lipid homeostasis, the improvement of antioxidant ability, and increased ATP synthesis in L-carnitine treated cells (Li et al., 2012). Previous study in rats found that the rate of gene transcription and activity of CPT1A also related to carnitine levels (Karlic et al., 2002). The present experiment with laying duck breeders showed that dietary Lys had significant effects on liver TG content and expression of the key fatty acid metabolic factors *PPARα* and *CPT1A*. The lowest liver TG content and highest *PPARα* and *CPT1A* expression were found with 8.8 g/kg Lys, which probably helped explain the lower abdominal fat content with this diet. ApoVLDL-II, a major component of the yolk protein precursors, only synthesized in female birds' liver, and VLDL is the major porter of TG from liver to the developing oocyte (Schneider et al., 1990; Walzem et al., 1999). Dietary deficiency of essential amino acids lead to decreased estrogen levels due to reduced ovarian activity (Narita et al., 2011). In this study, expression of *SREBP1* and *FAS* (*de novo* lipogenesis) was unaffected by dietary Lys in duck breeders, whereas the hepatic expression of *ApoVLDL-II* increased with increasing Lys, likely indicating increased capacity for VLDL secretion in laying duck breeders. These results suggest that Lys promotes lipolysis, increase VLDL secretion, and reduced hepatic TG in the breeders. Excessive deposition of VLDL leading to lipid metabolic disorders is the major cause of fatty liver (Kawano and Cohen, 2013). VLDLR is known as a member of the LDL receptor superfamily, plays an important role in hepatic lipid disorders, and its overexpression can increased liver TG content (Wang et al., 2014). In the present study, there was substantial reduction in relative abundance of *VLDLR* transcripts in liver of duck breeders with increasing dietary Lys. There was also decreased hepatic expression of *VLDLR* in hatchlings. Interestingly, the transcription of *FAS*, *HMGCR*, and *CPT1A* in hatchlings was also suppressed by increased maternal Lys, while *ApoVLDL-II* transcripts were not detected in hatchlings, probably because gene expression, production, and secretion of this protein are associated with oocyte development in avian species (Kidd et al., 2005; Yen et al., 2005; Yang et al., 2013). During embryonic development, approximately 50% of the initial yolk lipid is oxidized for energy production, the other 50% is incorporated into the body tissue and residual yolk of hatchlings. Therefore, Lys supplementation of duck breeders' diets increased lipid mobilization, and reduced lipolysis in hatchlings. The related mechanisms deserve further investigation.

CONCLUSIONS

In conclusion, the present study with laying duck breeders demonstrated that the productive and reproductive performance was improved by increasing dietary Lys to approximately 8.6 to 9.1 g/kg. The optimal Lys levels for maximizing egg production, egg weight, egg mass, FCR, and hatchability were 8.8, 8.6, 9.0, 9.1, and 9.1 g/kg, respectively. Dietary Lys concentrations affected expression of genes related to protein and lipid metabolism in laying duck breeders, and it probably improved the mobilization of protein and lipid in the hatchlings, which may be reflected in their performance.

ACKNOWLEDGMENTS

The authors sincerely thank W. Bruce Currie (Emeritus Professor, Cornell University, Ithaca, NY) for his help in revising the manuscript. This study was supported by the Earmarked Fund for Modern Agro-industry Technology Research System (CARS-42-13, CARS-42-15), the Supporting Program for the Research of State Key Laboratory of Livestock and Poultry Breeding, the National Key Research and Development Program (2018YFD0501504), the Science and Technology Program of Guangdong Province (2016A020210043), the Key Project of the Science and Technology Program of Guangzhou City (201804020091, 201904020001), the Science and Technology Program for Pearl River Nova of Guangzhou City (201710010159), the National Natural Science Foundation of China (31802082), the Supporting Program for the Research of State Key Laboratory of Livestock and Poultry Breeding, the Supporting Program for Guangdong Agricultural Research and Development Center of Livestock and Poultry Healthy Breeding, and the President Foundation of Guangdong Academy of Agricultural Sciences (201619).

REFERENCES

Baker, D. H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. BioKyowa Technol. Rev. 9:1–24.

Bechet, D., A. Tassa, D. Taillandier, L. Combaret, and D. Attaix. 2005. Lysosomal proteolysis in skeletal muscle. Int. J. Biochem. Cell Biol. 37:2098–2114.

Braun, C. M., J. R. Burgess, and M. A. Latour. 2001. Liver lipid accumulation in duck embryos and hatchlings change with parental age1. Neonatology 80:228–234.

Carew, L., J. McMurtry, and F. Alster. 2005. Effects of lysine deficiencies on plasma levels of thyroid hormones, insulin-like growth factors I and II, liver and body weights, and feed intake in growing chickens. Poult. Sci. 84:1045–1050.

Chang, L., P. Xie, Z. Bu, Q. Wang, S. Fu, and C. Mu. 2018. Effect of dietary lysine level on performance, egg quality and serum

biochemical indices of laying pigeons. J. Appl. Poult. Res. 27:152–158.
Ciacciariello, M., and N. C. Tyler. 2013. The effects of maternal dietary lysine intake on offspring performance to 21 days of age. J. Appl. Poult. Res. 22:238–244.
Corradetti, M. N., and K. Guan. 2006. Upstream of the mammalian target of rapamycin: do all roads pass through mTOR? Oncogene 25:6347–6360.
Del Vesco, A. P., E. Gasparino, D. O. Grieser, V. Zancanela, D. M. Voltolini, A. S. Khatlab, S. E. F. Guimaraes, M. A. M. Soares, and A. R. O. Neto. 2015. Effects of methionine supplementation on the expression of protein deposition-related genes in acute heat stress-exposed broilers. PLoS One 10:e0115821.
Deng, H., A. Zheng, G. Liu, W. Chang, S. Zhang, and H. Cai. 2014. Activation of mammalian target of rapamycin signaling in skeletal muscle of neonatal chicks: Effects of dietary leucine and age. Poult. Sci. 93:114–121.
Dozier, W. A., A. Corzo, M. T. Kidd, P. B. Tillman, and S. L. Branton. 2009. Digestible lysine requirements of male and female broilers from fourteen to twenty-eight days of age. Poult. Sci. 88:1676–1682.
Edwards, H. M., S. R. Fernandez, and D. H. Baker. 1999. Maintenance lysine requirement and efficiency of using lysine for accretion of whole-body lysine and protein in young chicks. Poult. Sci. 78:1412–1417.
Ekmay, R. D., C. Salas, J. England, S. Cerrate, and C. N. Coon. 2013. The effects of age, energy and protein intake on protein turnover and the expression of proteolysis-related genes in the broiler breeder hen. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 164:38–43.
Ekmay, R. D., C. Salas, J. England, S. Cerrate, and C. N. Coon. 2014. Lysine partitioning in broiler breeders is not affected by energy or protein intake when fed at current industry levels. Poult. Sci. 93:1737–1744.
Fernandez, S. R., S. Ayogi, Y. Han, C. M. Parsons, and D. H. Baker. 1994. Limiting order of amino acids in corn and soybean meal for growth of the chick. Poult. Sci. 73:1887–1896.
Folch, J., M. Lees, and G. Sloane-Stanly. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.
Fouad, A. M., W. Chen, D. Ruan, S. Wang, W. Xia, and C. Zheng. 2018. Effects of dietary lysine supplementation on performance, egg quality, and development of reproductive system in egg-laying ducks. J. Appl. Anim. Res. 48:386–391.
Grisoni, M. L., G. Uzu, M. Larbier, and P. A. Geraert. 1991. Effect of dietary lysine level on lipogenesis in broilers. Reprod. Nutr. Dev. 31:683–690.
Hevia, P., and W. J. Visek. 1980. Liver and serum lipids and lipoproteins of rats fed 5% L-lysine. Lipids 15:95–99.
Hiramoto, K., T. Muramatsu, and J. Okumura. 1990. Effect of methionine and lysine deficiencies on protein synthesis in the liver and oviduct and in the whole body of laying hens. Poult. Sci. 69:84–89.
Huang, X., C. Li, X. Zhang, P. He, G. Jiang, X. Wang, and Q. Dai. 2016. Effects of dietary lysine level on performance, egg quality, serum biochemical indices and nitrogen metabolism of *Linwu* ducks in peak laying period. Chin. J. Anim. Nutr. 28:410–417.
Kakhki, R. A. M., A. Golian, and H. Zarghi. 2016. Effect of dietary digestible lysine concentration on performance, egg quality, and blood metabolites in laying hens. J. Appl. Poult. Res. 25:506–517.
Karlic, H., S. Lohninger, T. Koeck, and A. Lohninger. 2002. Dietary L-carnitine stimulates carnitine palmitoyltransferases in the liver of aged rats. J. Histochem. Cytochem. 50:205–212.
Kawano, Y., and D. E. Cohen. 2013. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. J. Gastroenterol. 48:434–441.
Kersten, S. 2008. Peroxisome proliferator activated receptors and lipoprotein metabolism. PPAR Res. 2008:132960.
Kidd, M. T., C. D. Mcdaniel, E. D. Peebles, S. J. Barber, A. Corzo, S. L. Branton, and J. C. Woodworth. 2005. Breeder hen dietary L-carnitine affects progeny caecase traits. Br. Poult. Sci. 46:97–103.
Kim, H., M. Miyazaki, and W. C. Man. 2002. Sterol regulatory element-binding proteins (SREBPs) as regulators of lipid metabolism. Ann. N. Y. Acad. Sci. 967:34–42.
Kumair, K. N. R., V. R. Reddy, V. C. Preetham, D. S. Kumar, A. R. Sen, and S. V. R. Rao. 2016. Effect of supplementation of crystalline lysine on the performance of WL layers in tropics during summer. Trop. Anim. Health Prod. 48:705–710.
Lecker, S. H., A. L. Goldberg, and W. E. Mitch. 2006. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. JASN 17:1807–1819.
Li, J. L., Q. Y. Wang, H. Y. Luan, Z. C. Kang, and C. B. Wang. 2012. Effects of L-carnitine against oxidative stress in human hepatocytes: involvement of peroxisome proliferator-activated receptor alpha. J. Biomed. Sci. 19:32.
Lima, H. J. D., S. L. T. Barreto, J. L. Donzele, G. S. Souza, R. L. Almeida, I. F. F. Tinoco, and L. F. T. Albion. 2016. Digestible lysine requirement for growing Japanese quails. J. Appl. Poult. Res. 25:483–491.
Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408.
McGarry, J. D., and N. Brown. 1997. The mitochondrial carnitine palmitoyltransferase system-from concept to molecular analysis. Eur. J. Biochem. 244:1–14.
Mejia, L., C. D. McDaniel, and A. Corzo. 2012. Dietary influence of digestible lysine concentration on Cobb 500 hen broiler breeder reproductive performance. Poult. Sci. 91:426–431.
Mejia, L., C. D. McDaniel, M. T. Kidd, K. Lopez, and A. Corzo. 2013. Evaluation of carryover effects of dietary lysine intake by Cobb 500 broiler breeder hens. Poult. Sci. 92:709–718.
Meloche, K. J., B. I. Fancher, D. A. Emmerson, S. F. Bilgili, and W. A. Dozier. 2018. Effects of reduced digestible lysine density on myopathies of the Pectoralis major muscles in broiler chickens at 48 and 62 days of age1. Poult. Sci. 97:3311–3324.
Moran, E. T., and S. F. Bilgili. 1990. Processing losses, carcass quality, and meat yields of broiler chickens receiving diets marginally deficient to adequate in lysine prior to marketing. Poult. Sci. 69:702–710.
Narita, K, K. Nagao, M. Bannai, T. Ichimaru, S. Nakano, T. Murata, T. Higuchi, and M. Takahashi. 2011. Dietary deficiency of essential amino acids rapidly induces cessation of the rat estrous cycle. PLoS One 6:e28136.
National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
Prochaska, J. F., J. B. Carey, and D. J. Shafer. 1996. The effect of L-lysine intake on egg component yield and composition in laying hens. Poult. Sci. 75:1268–1277.
Rabie, M. H., and M. Szilágyi. 1998. Effects of L-carnitine supplementation of diets differing in energy levels on performance, abdominal fat content, and yield and composition of edible meat of broilers. Br. J. Nutr. 80:391–400.
Ruan, D., A. M. Fouad, Q. Fan, W. Xia, S. Wang, W. Chen, C. Lin, Y. Wang, L. Yang, and C. Zheng. 2018. Effects of dietary methionine on productivity, reproductive performance, antioxidant capacity, ovalbumin and antioxidant-related gene expression in laying duck breeders. Br. J. Nutr. 119:121–130.
Schmeisser, D. D., F. A. Kummerow, and D. H. Baker. 1983. Effect of excess dietary lysine on plasma lipids of the chick. J. Nutr. 113:1777–1783.
Schneider, W. J., R. Carroll, D. L. Severson, and J. Nimpf. 1990. Apolipoprotein VLDL-II inhibits lipolysis of triglyceride-rich lipoproteins in the laying hen. J. Lipid Res. 31:507–513.
Sibbald, I. R., and M. S. Wolynetz. 1986. Effects of dietary lysine and feed intake on energy utilization and tissue synthesis by broiler chicks. Poult. Sci. 65:98–105.
Souza, H. R. B., D. E. Faria, and V. C. Caetano. 2014. Digestible lysine levels for brown layers. Acta Sci. Anim. Sci. 36:369–372.
Tanphaichitr, V. T., M. S. Zaklama, and H. P. Broquist. 1976. Dietary lysine and carnitine: relation to growth and fatty livers in rats. J. Nutri. 106:111–117.
Tesseraud, S., R. Peresson, J. Lopes, and A. M. Chagneau. 1996. Dietary lysine deficiency greatly affects muscle and liver protein turnover in growing chickens. Br. J. Nutr. 75:853–865.
Tesseraud, S., I. Bouvarel, A. Collin, E. Audouin, S. Crochet, I. Seiliez, and C. Leterrier. 2009. Daily variations in dietary

lysine content alter the expression of genes related to proteolysis in chicken pectoralis major muscle. J. Nutr. 139:38–43.

Tian, D. L., R. J. Guo, Y. M. Li, P. P. Chen, B. B. Zi, J. J. Wang, R. F. Liu, Y. N. Min, Z. P. Wang, Z. Y. Niu, and F. Z. Liu. 2019. Effects of lysine deficiency or excess on growth and expression of lipid metabolism genes in slow-growing broilers. Poult. Sci. pez041, 98:2927–2932.

Urdaneta-Rincon, M., and S. Leeson. 2004. Muscle (pectoralis major) protein turnover in young broiler chickens fed graded levels of lysine and crude protein. Poult. Sci. 83:1897–1903.

Walzem, R. L., R. J. Hansen, D. L. Williams, and R. L. Hamilton. 1999. Estrogen induction of VLDLy assembly in egg-laying hens. J. Nutri. 129:467S–472S.

Wang, Z., X. Dou, S. Li, X. Zhang, X. Sun, Z. Zhou, and Z. Song. 2014. Nuclear factor (erythroid-derived 2)-like 2 activation-induced hepatic very-low-density lipoprotein receptor overexpression in response to oxidative stress contributes to alcoholic liver disease in mice. Hepatology 59:1381–1392.

Yang, S., Y. Suh, Y. M. Choi, S. Shin, J. Y. Han, and K. Lee. 2013. Loss of fat with increased adipose triglyceride lipase-mediated lipolysis in adipose tissue during laying stages in quail. Lipids 48:13–21.

Yen, C. F., Y. N. Jiang, T. F. Shen, I. M. Wong, C. C. Chen, K. C. Chen, W. C. Chang, Y. K. Tsao, and S. T. Ding. 2005. Cloning and expression of the genes associated with lipid metabolism in Tsaiya ducks. Poult. Sci. 84:67–74.