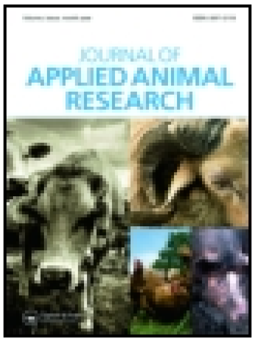

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Effects of dietary lysine supplementation on performance, egg quality, and development of reproductive system in egg-laying ducks

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ABSTRACT

This study was conducted to examine five lysine (Lys) levels (0.75, 0.80, 0.85, 0.90, and 0.95%) to estimate the optimal level of Lys for Longyan laying ducks from 22 to 38 wk of age. Nine hundred Longyan ducks aged 22 wk were assigned randomly to the 5 dietary treatments, where each treatment comprised 6 replicate pens with 30 ducks per pen. Lys had no effects on egg production, egg mass, feed conversion ratio, shape index, Haugh unit, yolk colour, yolk weight, or albumen weight, but the egg weight was improved significantly ($P < 0.05$) by dietary Lys supplementation, whereas the eggshell thickness, eggshell weight, and eggshell proportion decreased significantly as the Lys levels increased. Dietary supplementation of Lys did not affect the reproductive organ indices or the plasma levels of estradiol, luteinizing hormone, or follicle-stimulating hormone, whereas the plasma level of progesterone declined significantly ($P < 0.01$) as elevation the concentrations of Lys in the diets. In conclusion, the results indicate that the optimal Lys level in Longyan laying ducks diets is 0.80% to produce eggs with normal egg weight without adverse effects on the eggshell weight, eggshell proportion, or eggshell thickness.

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Lysine; laying ducks; performance; egg quality

1. Introduction

In avian species, the second limiting amino acid, lysine (Lys), is an indispensible amino acid (Schutte & Smink 1998) because it is required to maintain the normal physiological functions of immune system (Kidd 2004), improve intestinal functions (Vaezi et al. 2011), regulate protein and lipid metabolism (Tesseraud et al. 1996; Fouad & El-Senousey 2014), maximize the productive performance (Tesseraud et al. 1999; Bons et al. 2002; Lemme et al. 2002; Mehri et al. 2013), and increase consumer acceptance through enhancing the product quality (Leclercq 1998). Deficiency of Lys induces a significant reduction in immune organs development (spleen and bursa of fabricius) (Mulyantini 2014), antibody synthesis (Mahdavi et al. 2012), feather growth (Lima et al. 2016), alter secretion of thyroid gland hormones, growth hormones (Carew et al. 1997, 2005; Wang et al. 2006), impairs growth performance, feed efficiency, egg yield, egg mass, carcass yield, protein accretion (Kidd & Fancher 2001; Faria et al. 2003; Dozier et al. 2008, 2009; Dozier & Payne 2012; Lima et al. 2016), and promote abdominal fat deposition (undesirable fat) which lower meat quality and consumers' acceptance (Fouad & El-Senousey 2014). Additionally, feeding birds diet containing inadequate concentration of Lys negatively affected bone structure and its development (Latshaw 1993; Sekine et al. 1996; Lima et al. 2016). Dietary Lys supplementation enhances cellular and humoral immune responses in broiler chickens (Chen et al. 2003; Panda et al. 2011; Taghinejad-Roudbaneh et al. 2011). Studies with commercial broiler chickens (Faridi et al. 2015), local breed of chickens (Nasr & Kheiri 2011; Yuan et al. 2015), quails (Mehri et al. 2013), turkeys (de Paula Dorigam et al. 2016), White Pekin ducks (Xie et al. 2009), and local Korean ducks (Wickramasuriya et al. 2016) suggested increasing Lys levels to improve average daily gain, feed consumption, and feed efficiency. Moreover, carcass yield, breast meat yield, water-holding capacity, tenderness, and meat taste improved as a result of increasing Lys concentrations, whereas abdominal fat and cooking loss declined which led to enhance meat quality and consumer acceptance (Berri et al. 2008; Watanabe et al. 2015; Zhai et al. 2016).

Data regarding the effects of dietary Lys supplementation on avian species including broiler breeders, broiler chickens, meat-type ducks, quails, turkeys, and laying hens have been available (Dozier et al. 2008, 2009; Xie et al. 2009; Dozier & Payne 2012; Mehri et al. 2015; de Paula Dorigam et al. 2016; Kakhki et al. 2016; Wickramasuriya et al. 2016), but to the best of our knowledge, almost no data are available regarding the effects of dietary Lys supplementation on productive performance, egg quality, reproductive organ indices, and reproductive hormones in laying ducks. Therefore, the main goals of this study were to investigate the effects of dietary Lys supplementation on laying

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ducks performance, egg quality, reproductive organ indices, and reproductive hormones.

2. Materials and methods

2.1. Animals and diet

All of the experimental procedures were revised and approved by the Animal Care and Use Committee of Guangdong Academy of Agriculture Science, China. A total of 900 Longyan ducks (a typical breed of egg-laying ducks in South China) with similar body weights were placed randomly to five dietary treatments, that is, six replicate pens (each replicate was placed with an indoor area of 3 × 1.8 m, outdoor area of 3 × 1.8 m, and pool area of 3 × 1.8 m), and they were studied from 22 to 38 wk of age. The same basal diet in pellet form (Table 1), which was supplemented with 0.0 (control), 0.064, 0.128, 0.192, or 0.256% of Lys in the form of L-Lys monohydrochloride (98.5% L-Lys, Shandong Shouguang Juneng Golden Corn Co. Ltd, Shandong, People's Republic of China) was offered. The control diet was prepared to cover the nutritional requirements of Longyan egg-laying ducks (Fouad et al. 2016), with the exception of Lys. Fresh water was offered *ad libitum*, while feed was introduced twice daily with an average of 160 g/bird/d without residue. The ducks had access to the outdoor and pool areas during the daytime, but they were housed indoors at night. The light was naturally during the daytime with 4 h of incandescent lighting at 15 lx from 1830 to 2230.

2.2. Performance and egg quality

Feed consumption and egg production were registered daily. Eggs were harvested and individually weighed and graded daily on a replication basis. Feed conversion ratio (FCR) was calculated as grams of feed per gram of egg mass daily on a replication basis and then presented as the averages for the complete 16-wk study period. Two eggs were collected at random from each replicate at the end of the experimental period to determine egg quality (egg shape index, Haugh unit, yolk colour, eggshell thickness) as described by Fouad et al. (2017).

Table 1. Composition of the basal diet and nutrient levels.

Ingredients	Value (%)
Corn	54.48
Soybean meal	19.7
Wheat bran	9.49
Corn gluten	5.0
Limestone	8.485
Calcium hydrogen phosphate	1.33
DL-methionine	0.13
Zeolite powder	0.085
Sodium chloride	0.3
Premix[a]	1.0
Total	100.0
Nutritional value	
AME (Kcal/kg)	2500
Crude protein	17.0
Calcium	3.6
Total P	0.59
Available phosphorus	0.35
Total lysine	0.75
Total methionine	0.40

[a]Supplied per kilogram of diet: retinyl palmitate, 12,000 IU; cholecalciferol, 2000 IU; DL-α-tocopheryl acetate, 38 mg; menadione sodium bisulphite, 1.0 mg; thiamin mononitrate, 3.0 mg; riboflavin, 9.6 mg; pyridoxine hydrochloride, 6.0 mg cobalamin, 0.03 mg; chloride choline, 500 mg; nicotinic acid, 25 mg; calcium-D-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, 0.5 mg; Se, 0.4 mg.

2.3. Blood sample collection

At the end of the experiment, two ducks from each replicate were randomly selected for blood sampling. Five millilitres of blood was collected from the wing vein using heparinized tubes. Blood was centrifuged at (1200 × *g*) at 4°C for 10 min to harvest plasma, then plasma was stored at −20°C until estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone (P4) (reproductive hormones) determined as mentioned by Xia et al. (2017).

2.4. Reproductive organ indices

At the end of the experiment after blood samples were taken, the ducks were slaughtered to harvest ovaries, small yellow follicles (SYFs; 3 mm <diameter <8 mm) and large follicles (LYFs; diameter >8 mm). The ovaries, SYFs, and LYFs weighed using an electronic balance then the numbers of SYFs and LYFs were registered.

2.5. Statistical analysis

Data were analysed as a single factor design using one-way ANOVA of SPSS 16.0 software (SPSS Inc., Chicago, IL). The linear and quadratic effects of Lys among treatments were analysed using a contrast statement. Significant differences among treatments were tested using Tukey post hoc test at a significance level of $P < 0.05$.

3. Results and discussion

3.1. Productive performance

The egg production, egg mass, and FCR were not affected significantly ($P > 0.05$) by Lys supplementation, but the egg weight increased linearly ($P < 0.05$) as a result of increasing Lys concentration (Table 2). These findings are in agreement with an earlier study of laying hens by Prochaska et al. (1996), who reported that increasing Lys levels in Hy-Line W-36 laying hens diets from 0.70% to 1.58% at 42 wk of age for 22 wk had no effect on their egg production, whereas their egg weight increased significantly as dietary Lys level increased. Schutte and Smink (1998) found that egg production, egg mass, egg weight, and FCR of White Lohmann laying hens did not change by increasing Lys concentration in their diets from 0.65 to 0.93 for 12 wk during peak production, whereas Schmidt et al. (2008) observed that egg production, egg weight, and FCR of Lohmann White laying hens enhanced significantly as a result of increasing Lys level from 0.555% to 0.755% for 16 wk during the second cycle of production, while adding 0.2% Lys to the diets that containing 0.70% Lys in Lohmann LSL laying hens aged 24 wk for 20 wk did not affect egg weight (de Carvalho et al. 2015).

Table 2. Effects of dietary L-lysine on the performance of laying ducks.

	Dietary Lys (%)						Contrast *P*-value		
Trait	0.75	0.80	0.85	0.90	0.95	SEM[b]	Level	Linear	Quadratic
Egg production (%)	88.3	86.0	86.9	87.1	87.4	0.4	0.7	0.8	0.3
Egg weight (g)	65.1	66.0	66.4	65.1	66.9	0.2	0.03	0.057	0.9
Egg mass (g/d/d)	57.5	56.8	57.7	56.7	58.4	0.3	0.5	0.5	0.3
FCR[a]	2.79	2.82	2.77	2.82	2.74	0.02	0.5	0.5	0.3

[a]Feed conversion ratio, g of feed/g of egg mass
[b]Standard error of means, $n = 6$.

Faria et al. (2003) increasing the Lys concentrations from 0.60% to 0.76% in Hy-Line W-36 from 44 to 51 wk of age or from 58 to 65 wk of age under different ranges of ambient temperature did not affect their productive performance including laying rate, egg weight, egg mass, or FCR, while reducing Lys level from 0.60 to 0.48 significantly lowered laying rate, egg weight, and egg mass, and impaired FCR. Also, in Hy-Line W-36 age 26 wk, increasing Lys concentration from 0.66% to 0.94% in their diets for 16 wk improved FCR without significant effects on egg production, egg weight, or egg mass (Shahir et al. 2006). In addition, results of da Rocha et al. (2009) exhibited significant improvements in egg production, egg weight, egg mass, and FCR of Hy-Line W-36 laying hens aged 24 wk when they fed basal diet (0.545%Lys) supplemented with 0.059%, 0.118%, 0.177%, 0.236%, or 0.295% Lys for 16 wk. Jardim Filho et al. (2010) reported that egg production, egg weight, and FCR improved by increasing Lys level from 0.60% to 0.80% for 20 wk in Hy-Line W-36 aged 25 wk, whereas Figueiredo et al. (2012) observed that increasing Lys level from 0.675% to 0.879% in Hy-Line W-36 laying hens age 42 wk for 16 wk did not modulate egg production, but egg weight, egg mass, and FCR improved as a result of increasing Lys levels. Moreover, Kakhki et al. (2016) reported that feeding Hy-Line W-36 from 32 to 40 wk of age diet containing 0.807% Lys improved egg production, egg weight, egg mass, and FCR compared with control group (0.657% Lys), whereas results of Souza et al. (2014) in Hy-Line brown laying hens showed that egg production, egg weight, egg mass, and FCR did not improve by feeding them diets containing different levels of Lys (0.70%, 0.75%, 0.80%, 0.85%, or 0.95%) from 25 to 41 wk of age, while in Dekalb White laying hens, Silva et al. (2015) found that increasing the Lys level from 0.64% to 0.91% from 32 to 48 wk of age had no effects on egg production or FCR, but the egg weight improved significantly. Additionally, results of Kumari et al. (2016) showed that egg production, egg weight, egg mass, and FCR did not respond to increasing Lys level (from 0.60% to 0.7%) in laying hens diets that containing different levels of crude protein. Similarly, Hurtado Nery et al. (2015) noted that increasing Lys levels from 0.916% to 1.276% in laying quail diets for 26 wk had no significant effect on egg production, egg weight, or FCR, while Ribeiro et al. (2013) found that elevating Lys levels from 0.95% to 1.20% for 12 wk showed significant improvements in egg weight, egg mass, and FCR without significant effect on egg production in laying quails. In quails, also, Costa et al. (2008) recorded a significant increase in egg production without any change in egg weight or FCR due to increasing Lys levels from 0.880% to 1.040%, whereas Lima et al. (2016) observed significant improvements in egg production, egg mass, and FCR by increasing Lys levels from 0.88% to 1.28% for 8 wk, but egg weight did not respond to increase Lys levels, while Pinto et al. (2003) found that increasing Lys concentrations from 0.890% to 1.300% enhanced egg weight, egg mass, FCR without effects on egg production.

In native Korean ducklings, increasing Lys levels from 0.52% to 1.22% during starter phase enhanced body weight gain and FCR (Wickramasuriya et al. 2016). Also, in White Pekin ducks aged 1 wk, increasing Lys concentrations from 0.65% to 1.25% significantly improved average daily gain, FCR, and meat yield at 21 d of age (Xie et al. 2009). Similarly, in growing quails, elevating levels of Lys from 0.84% to 1.29% increased body weight gain and improved FCR (Mehri et al. 2015). Moreover, feeding turkey toms diets supplemented with Lys improved body weight gain and FCR compared with the control group (deficient in Lys) (Lemme et al. 2002). In broiler chickens, FCR was impaired due to feeding them diets contained less Lys than recommended by NRC, 1994, for 10 d (Watanabe et al. 2017). The body weight gain and FCR of broiler chickens age 1 d old were improved significantly as a result of increasing Lys levels in their diet from 0.88% to 1.43% for 18 d (Kidd & Fancher 2001), whereas increasing Lys concentrations in broiler chicken diets aged 14 d from 1.1% to 1.65% for 10 d did not affect body weight gain or FCR (Watanabe et al. 2015). Also, feeding broiler chickens aged 1 d diets contained 1.73% or 3.25% Lys for 2 wk did not affect their productive performance compared with the diets that contained 1.25% Lys, while feeding broiler chickens aged 14 d diets contained 1.25% Lys for 13 d enhanced their productive performance compared with the diets that contained 1.07% Lys (Kiess et al. 2013). It is clear from previous studies in egg-type birds and meat-type birds that effects of dietary Lys supplementation on productive performance differ according to Lys level in the basal diet (basal diet is deficient in Lys or not), bird age, experimental period, and/or bird strain.

3.2. Egg quality

As shown in Table 3, dietary Lys supplementation had no effects on egg shape index, Haugh unit, yolk colour, yolk weight, yolk proportion, albumen weight, albumen promotion, or eggshell strength, whereas eggshell weight, eggshell proportion, and eggshell thickness declined linearly ($P < 0.05$) with increasing Lys levels. Kakhki et al. (2016) reported that increasing Lys levels from 0.657% to 0.857% in Hy-Line W36 laying hens did not affect egg shape index, yolk proportion, albumen proportion, eggshell proportion, or eggshell thickness, whereas Haugh unit enhanced with elevating Lys levels in their diets, while Kumari et al. (2016) found that Hugh unit, yolk colour, and eggshell thickness of WL laying hens did not alter by increasing Lys concentration in their diets. Also, in Hy-Line Brown laying hens and Hy-Line W-36 laying hens, the findings

Table 3. Effects of dietary L-lysine on egg quality.

	Dietary Lys (%)						Contrast *P*-value		
Trait	0.75	0.80	0.85	0.90	0.95	SEM[a]	Level	Linear	Quadratic
Egg shape index	72.2	73.1	73.2	73.5	72.2	0.3	0.7	0.3	0.7
Haugh units	68.2	70.0	71.9	69.5	75.3	1.6	0.6	0.2	0.8
Yolk colour	8.6	8.7	8.5	8.8	8.7	0.1	0.7	0.5	0.9
Yolk weight (g)	20.5	21.4	21.7	21.5	21.9	0.2	0.4	0.1	0.5
Yolk (%)	31.6	32.4	32.6	33.0	32.7	0.3	0.7	0.3	0.4
Albumin weight (g)	36.5	36.6	36.8	35.5	37.5	0.2	0.2	0.4	0.3
Albumin (%)	56.1	55.4	55.4	55.0	56.1	0.3	0.8	0.9	0.2
Eggshell thickness (mm)	0.324	0.323	0.322	0.312	0.299	0.003	0.01	0.002	0.4
Eggshell strength (N)	4.4	4.5	4.4	4.1	4.1	0.1	0.6	0.2	0.8
Eggshell weight (g)	8.0	8.0	7.8	7.7	7.5	0.07	0.1	0.03	0.3
Eggshell (%)	12.3	12.1	11.9	11.9	11.2	0.1	0.04	0.005	0.3

[a]Standard error of means, $n = 12$.

Table 4. Effects of dietary L-lysine on reproductive organ indices of laying ducks.

	Dietary Lys (%)						Contrast *P*-value		
Trait	0.75	0.80	0.85	0.90	0.95	SEM[a]	Level	Linear	Quadratic
Oviduct length (cm)	52.9	51.6	54.7	51.8	49.1	0.9	0.7	0.3	0.7
Oviduct weight (g)	39.7	37.5	40.6	39.2	39.6	0.6	0.6	0.2	0.8
Ovarian weight (g)	46.3	45.8	47.9	48.1	49.7	0.9	0.7	0.5	0.9
Large follicles number	5.1	4.5	5.3	5.5	5.2	0.2	0.4	0.1	0.5
Small follicles number	32.5	31.0	33.6	44.0	28.8	1.7	0.7	0.3	0.4

[a]Standard error of means, $n = 12$.

exhibited no improvements in the Haugh unit, yolk weight, yolk%, albumen weight, albumen%, eggshell weight, eggshell%, or eggshell thickness when they consumed diets supplemented with Lys (da Rocha et al. 2009; Souza et al. 2014), while de Carvalho et al. (2015) and Schmidt et al. (2008) found a significant decline in eggshell percentage due to increasing Lys levels in Lohmann LSL laying hens diets, whereas yolk and albumen percentage did not change. In laying quails, Ribeiro et al. (2013) recorded a significant increase in yolk and albumen weight when they consumed diets containing 1.1% Lys compared with the control group that consumed diets containing 0.95% Lys, whereas results of Costa et al. (2008) showed that albumen, yolk and eggshell weight and their proportion did not change by increasing Lys levels, while Pinto et al. (2003) reported a significant reduction in eggshell proportion as a result of increasing Lys levels from 0.890% to 1.300%. The findings of previous studies indicate that the effects of the Lys levels on egg quality is variable, which may be related to level of Lys in the basal diet, age, strain, and/or experimental period. Increasing egg weight which induced by increasing Lys levels requires an increase in eggshell weight as a result of increasing calcium deposition, but eggshell weight declined because increasing Lys levels did not alter serum Ca^{2+} concentrations (Panda et al. 2011) due to the available amount of calcium to produce eggshell for an egg in egg-laying birds is limited (2.0–2.5 g Ca^{2+}) regardless its egg size (egg weight) (Jonchère et al. 2012). This may clarify why increasing the Lys levels led to a significant reduction in eggshell weight and its thickness.

3.3. Oviduct, ovary-related indices, and reproductive hormones

Table 4 shows that the oviduct length, weight, ovary weight, and number of large and small follicle were not significantly ($P > 0.05$) affected by dietary Lys supplementation. Also, the E2, LH, FSH concentrations in the plasma of laying ducks were not significantly affected by Lys levels, whereas the concentration of P4 was decreased significantly as Lys levels increased (Table 5). The oviduct length, weight, ovary weight, and number of large and small follicle are used to judge ovarian development and its physiological functions (Sun et al. 2015; Meng et al. 2016; Xia et al. 2017). The reproductive hormones including E2, LH, FSH, and P4 are associated with development of reproductive organs (Zhang et al. 1997). To the best of our information, our experiment is the first experiment to evaluate the effects of dietary Lys supplementation on reproductive organ indices and reproductive hormones. Therefore, it is very difficult to compare our findings with others. However, increasing the levels of Arginine (an essential amino acid) in laying ducks diets from 17 to 31 wk did not affect the levels of E2, LH, or

Table 5. Effects of dietary L-lysine on reproductive hormones of laying ducks.

	Dietary Lys (%)						Contrast *P*-value		
Trait	0.75	0.80	0.85	0.90	0.95	SEM[a]	Level	Linear	Quadratic
E2 (pg/mL)	349.3	257.6	358.4	338.3	263.5	23.5	0.5	0.6	0.6
LH (mIU/mL)	2.1	2.2	2.2	2.1	1.8	0.1	0.8	0.3	0.5
FSH (mIU/mL)	2.5	2.8	2.7	2.9	2.5	0.1	0.4	0.9	0.1
Progesterone (pg/mL)	348.3	305.0	262.5	270.8	227.5	15.6	0.1	0.01	0.7

Note: E2, estradiol-17β; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
[a]Standard error of means, $n = 12$.

FSH hormone, whereas the concentration of P4 decreased and the number of small follicle increased (Xia et al. 2017). Also, Meng et al. (2016) observed that increasing the concentrations of methionine (the first limiting amino acid in poultry diets) from 0.26% to 0.39% in Chinese laying hens diets during grower phase significantly declined the concentrations of LH and FSH in serum and the account of large and small follicles. P4 is linked to egg production, where diminishing the concentration of P4 was associated with declining the percentage of egg production in laying hens (González-Morán 2016; Long et al. 2017). Therefore, further studies are needed to elucidate the influence of dietary Lys supplementation on development of reproductive system in egg-type birds.

4. Conclusion

In conclusion, increased the Lys levels improved egg weight, but reduced eggshell weight and eggshell thickness. Therefore, we suggest formulating diets containing 0.80%Lys for Longyan laying ducks to obtain the maximum egg weight without adverse effects on the eggshell weight and eggshell thickness.

Disclosure statement

No potential conflict of interest was reported by the authors.

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