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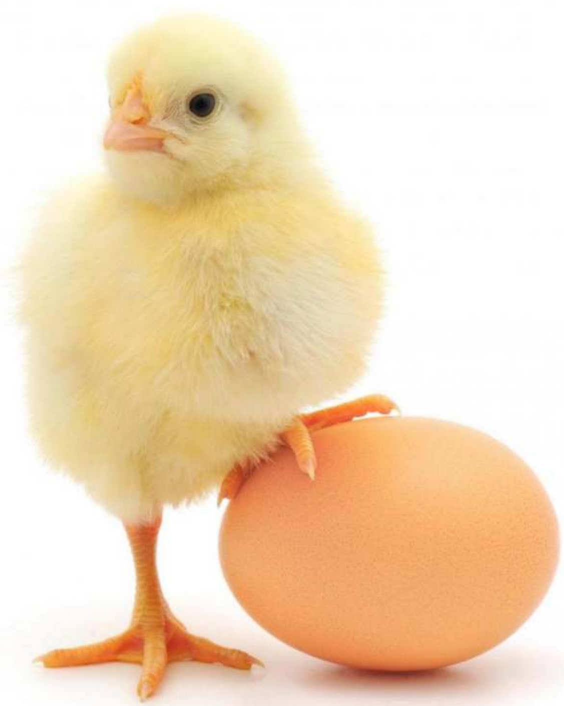

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Research Article
The Effects of Supplementing Lysophosphatidylcholine in Diet on Production Performance, Egg Quality and Intestinal Morphology of Laying Hens

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Abstract
Background and Objective: This study was conducted to evaluate the effects of supplementation of Lysophosphatidylcholine (LPC) in diets on production performance, egg quality and intestinal morphology of laying hens. **Materials and Methods:** A total of 288 commercial laying hens (Lohmann brown-classic) from 33-46 weeks of age were used in this study. The hens were divided into 3 groups of 8 replications with 12 hens each. According to the experimental groups, hens were fed as 1) control diet, 2) control diet with 0.05% LPC and 3) control diet with 0.10% LPC. **Results:** At the end of the trial, supplementations of LPC (0.05 and 0.10%) significantly improved feed conversion ratio (FCR), increased egg size, decreased feed intake and reduced feed cost per egg weight (FCE) (p<0.01), although significant effect on percentage of egg production, egg weigh and egg mass were not observed. Neither of internal and external egg qualities were influenced by LPC supplementation. In term of morphology, adding LPC significantly decreased villi height in the segment of duodenum and conversely increased in the jejunum (p<0.05). **Conclusion:** It is concluded that supplementation of LPC may improve nutrients utilization (digestion or/and absorption) via the modification of gut morphology.

Key words: Egg production, egg quality, intestinal morphology, laying hen diet, lysophosphatidylcholine (LPC)

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The egg yolk is a single massive cell weighing about 17-20 g in an average egg and is comprised of 51-52% water, 16-17% protein and 31-33% fat[1]. Fat is the largest nutritive component of egg yolk (65% triglyceride, 28% phospholipids and 7% cholesterol) and there are many biological functions of fat such as precursor of hormone, energy reserve, composition of cell membrane as well as fat soluble vitamins[2]. Therefore, the utilizations of fat (digestion, absorption and metabolism) in laying hen is important for egg production.

Lysophosphatidylcholine (LPC) is mono-acyl derivatives of phospholipids resulting from the action of phospholipase A2, which hydrolyse the ester bond at sn-2 position[3]. It is an important metabolite produced by many cells and are widely distributed in a variety of tissue and are capable of increasing ion permeation in membranes, alter mucosal barrier function[4] and modify the activity of various membrane associated enzymes[5]. In term of animal productions, supplementing LPC increased egg weight due to the increment of egg yolk weight of laying hens[2,6] improved body weight gain during starter period[7] and FCR of broiler chicks[8] improve broiler performance through increasing nutrient utilization[9,10], increased weight gain and nutrient digestibility of weaning pig[11,12]. Although, could not found any improvement in lipid digestibility during finishing period of pigs fed LPC[13].

During peak egg production period, the hens require high nutrients to support their productivity. The improvement of gut morphology by supplementing LPC may promote egg production performance of laying hens. Therefore, this study was conducted to evaluate the effect of supplementing LPC in diet on egg production, egg quality and intestinal morphology of laying hens.

MATERIALS AND METHODS

Animal and management: A total of 288 laying hens were used for 12 weeks (from 33-46 weeks of age) and kept under an evaporative cooling system to control air ventilation and temperature. The hens were divided into 3 experimental groups. Each group was consisted of 8 replications and 12 hens each. The hens were kept in wire cages with 4 hens per cage and the lighting program was set 16 h. Feed and water were offered *ad libitum*.

Experimental diets: The experimental diets were based on corn-soybean, all nutrient requirements were provided according to the Lohmann[14] Brown Management Guide. Feed ingredients and nutrients composition are presented in Table 1. The diets were assigned as (1) control diet (corn-soybean meal diet) (2) control diet with 0.05% LPC (3) control diet with 0.10% LPC. The Lysophosphatidylcholine (LPC) is offered from Devenish Nutrition (Belfast, North Ireland) and marketed under the trade name Lipidol® (50% lysophosphatidylcholine along with an inert calcium silicate carrier).

Egg production and egg quality: Egg production performance from 33 to 46 weeks of age were recorded. Percentage of egg production and egg weight were collected daily. The eggs are graded according to egg weight (Size 0 ≥70 g, Size 1 ≥65 g, Size 2 ≥60 g, Size 3 ≥55 g, Size 4 ≥50 g) at four days interval. Feed consumption and egg mass (g hen^{-1} day^{-1}) were used to calculate FCR (kg feed kg^{-1} egg mass). Feed cost per egg weight (FCE; Baht kg^{-1} egg) and mortality (%) were also determined.

Table 1: Feed ingredients of experimental diets and chemical composition of experimental diets

	LPC 0.00%	LPC 0.05%	LPC 0.10%
Corn	59.110	59.110	59.110
Soybean 48% CP	26.750	26.750	26.750
Palm oil	2.550	2.550	2.550
DL-Methionine	0.160	0.160	0.160
Calcium carbonate	8.950	8.950	8.950
MCP 21% P	1.240	1.240	1.240
Salt	0.110	0.110	0.110
Sodium bicarbonate	0.400	0.400	0.400
Premixes*	0.500	0.500	0.500
LPC	0.000	0.050	0.100
Total	100.000	100.050	100.100
Cost kg^{-1} (bath)	12.160	12.280	12.420
ME. for poultry (kcal kg^{-1})	2.737	2.737	2.737
Dry matter (%)	87.984	87.984	87.984
Crude protein (%)	17.000	17.000	17.000
Crude fat (%)	5.136	5.136	5.136
Crude fiber (%)	3.792	3.792	3.792
Lysine (%)	0.885	0.885	0.885
Met+Cys (%)	0.730	0.730	0.730
Methionine (%)	0.433	0.433	0.433
Threonine (%)	0.651	0.651	0.651
Valine (%)	0.825	0.825	0.825
Isoleucine (%)	0.736	0.736	0.736
Arginine (%)	1.123	1.123	1.123
Tryptophan (%)	0.187	0.187	0.187
Calcium (%)	3.730	3.730	3.730
Total phosphorus (%)	0.595	0.595	0.595
Avail. phosphorus (%)	0.380	0.380	0.380
Sodium (%)	0.270	0.270	0.270

*Premix: Consist of vitamin A: 5.0 MIU, D3 MIU: E 4,000 IU. K3: 0.6 g, B1: 0.8 g, B6: 1.2 g, B12: 0.0025 g, Nicotinic acid: 5.00 g, pantothenic acid: 3.76 g, Folic acid: 0.2 g, Biotin: 0.036 g, Mn: 24.00 g, Zn: 20.00 g, Fe: 16.00 g, Cu: 4.00 g, Iodine: 0.8 g, Co: 0.08 g, Se: 0.04 g and Carrier added to 1.00 kg premix

Four eggs from each replication that have weight close to the replication's mean were chosen twice a week to analyze egg qualities such as specific gravity, shell thickness (mm), albumen height (mm), albumen weight (g), yolk weight (g), eggshell weight (g), albumen (%), yolk (%), eggshell (%), albumen:yolk ratio, Haugh units and yolk color.

Intestinal morphology: Tissue samples of 32 hens were collected at the end of the experiment. One hen per replication was randomly selected from each pen and stunned with CO_2 and slaughtered and entire gastrointestinal tract was removed the stomach and selected three different locations of the small intestine. Tissue sample for morphological measurements were taken from the duodenum (5 cm from the pylorus), jejunum (5 cm posterior to the yolk stalk) and ileum (2 cm anterior to the ileocecal valve) rinsed in physiological saline, every sample about 2 cm in length fixed in phosphate-buffered formalin (10% formalin, pH = 7.6). Histological slides were prepared from 2 cross-sections (5 um thick) of each intestinal sample, processed in low-melt paraffin and stained with hematoxylin-eosin to evaluate morphological parameters[15].

Statistical analyses: Data were analyzed using ANOVA (two-way) in a completely randomized design. Differences among treatment means were tested for significance by using the Duncan's multiple range tests at 5% significance level[16].

RESULTS AND DISCUSSION

Productive egg performance: The effects of LPC on egg production performance are shown in Table 2 and 3. Percentage of egg production, body weight, egg weight and egg mass were not significantly affected by LPC supplementation. However, supplementing LPC (0.05 and 0.10%) in diet result in lower feed intake, FCR and feed cost per egg (FCE) ($p<0.01$). Moreover, the number of large size egg (Grade 0: ≥70 g) was significantly high (p = 0.05), while the medium size (Grade 2: 60-64 g) was low by LPC supplementations (Table 3).

Percentage of egg production, egg weight and body weight were not significantly influenced by LPC supplementations. These are in agreement with Han *et al.*[2] and Melegy *et al.*[17] who reported that LPC did not affect these productive traits. However, it is clear that LPC decrease feed intake and improve FCR. It can be implied that supplementing LPC may enhance digestibility and/or absorption of the nutrients. Accordingly, Han *et al.*[6] reported that supplementing LPC in diet improved FCR due to the increase of nutrients digestibility, decreased feed intake and increase egg weight. Also, Zaefarian *et al.*[18] found a significant decrease in feed intake in broilers fed diets containing 3.5 kg t^{-1} of LPC. In agreement with Han *et al.*[2] this study also found high number of large (≥72.9 g) size eggs with a concomitant reduction in the number of medium (53-62.9 g) size eggs when fed diet supplemented with LPC. It is possible that LPC may improve fat utilization and energy due to its emulsifying properties and/or increase of nutrient absorption via increasing micelle formations[6,11,19,20].

Egg quality: There were no significant effect of LPC on egg quality such as specific gravity, albumen height, shell weight,

Table 2: Effect of LPC supplementation in diet on egg production performance of laying hens

	LPC 0.00%	LPC 0.05%	LPC 0.10%	p-value	SEM
Initial weight(g)	1.958±67.75	1.961±35.54	1.957±39.30	0.99	5.67
Final weight (g)	2.001±51.47	2.008±36.23	2.005±36.11	0.89	4.32
Weight gain(g)	42.780±19.38	47.560±19.51	48.130±19.51	0.83	2.05
Egg production	91.700±3.57	93.890±4.28	95.300±2.56	0.15	0.76
Egg weight	67.250±2.26	67.900±1.60	67.450±1.76	0.78	0.37
Feed intake (g h^{-1} day^{-1})	$124.840±4.66^{A}$	$119.180±2.34^{B}$	$119.050±1.98^{B}$	<0.01	0.84
Egg mass	61.680±3.23	63.570±3.51	64.090±2.52	0.29	0.64
FCR	$2.030±0.07^{A}$	$1.880±0.11^{B}$	$1.860±0.07^{B}$	<0.01	0.02
FCE	$24.640±0.82^{A}$	$23.080±1.30^{B}$	$23.060±0.92^{B}$	0.01	0.25

^{A,B,C}Means within the same row without the same superscript letter are significant different ($p<0.05$), FCE: Feed cost per egg weight 1 kg

Table 3: Effect of LPC supplementation in diet on egg size of laying hens

	LPC 0.0%	LPC 0.05%	LPC 0.1%	p-value	SEM
Size 0	$28.06±9.04^{B}$	$39.87±11.99^{A}$	$39.69±8.64^{B}$	0.04	2.27
Size 1	38.06±7.30	38.21±7.70	32.20±4.79	0.15	1.44
Size 2	$29.70±9.10^{A}$	$19.62±7.02^{B}$	$23.35±5.10^{B}$	0.04	1.66
Size 3	4.00±4.60	2.21±1.92	3.83±2.88	0.51	0.67
Size 4	$0.18±0.27^{AB}$	$0.09±0.17^{B}$	$0.93±1.20^{A}$	0.05	0.16

Size 0: ≥70 g, Size 1: ≥65 g, Size 2: ≥60 g, Size 3: ≥55 g, Size 4: ≥50 g ^{A,B,C}Means within the same row without the same superscript letter are significant different ($p<0.05$)

Table 4: Effect of LPC supplementation in diet on egg quality of laying hens

	LPC 0.00%	LPC 0.05%	LPC 0.10%	p-value	SEM
Albumen height (mm)	6.49±0.44	6.40±0.35	6.47±0.36	0.89	0.08
Yolk weight (g)	16.15±0.50	16.55±0.29	16.26±0.33	0.13	0.08
Shell weight (g)	16.15±0.50	16.55±0.29	16.26±0.33	0.13	0.08
Albumen weight (g)	43.68±1.27	45.21±1.26	44.62±1.12	0.06	0.27
Albumen (%)	65.79±0.60	66.06±0.77	66.10±0.71	0.62	0.14
Yolk (%)	24.30±0.47	24.19±0.59	24.06±0.57	0.70	0.11
Shell (%)	9.93±0.16	10.19±0.95	9.83±0.18	0.43	0.11
Ratio Al: Yolk	2.72±0.08	2.74±0.10	2.75±0.09	0.72	0.02
Haugh units	77.48±2.61	76.42±2.56	77.20±2.46	0.69	0.50
Specific gravity	1.09±0.00	1.09±0.00	1.09±0.00	0.34	0.00
Strength (N)	4.01±0.16	4.31±0.66	3.91±0.23	0.16	0.09
Yolk color	8.64±0.10	8.60±0.08	8.54±0.10	0.14	0.02
Shell thickness (mm)	0.37±0.01	0.37±0.01	0.37±0.01	0.85	0.00

Table 5: Effect of LPC supplementation in diet on Intestinal morphology of laying hens

	LPC 0.00%	LPC 0.05%	LPC 0.10%	p-value	SEM
Villous height (µm)					
Duodenum	928.13±114.12^{A}	759.79±113.22^{B}	727.35±88.37^{B}	0.01	29.46
Jejunum	386.82±84.38^{B}	528.08±82.90^{A}	447.68±118.83^{A}	0.04	23.85
Ileum	255.34±109.24	268.81±131.69	238.83±46.76	0.86	21.38
Crypt depth (µm)					
Duodenum	184.71±67.72	170.98±40.96	148.63±31.67	0.40	10.72
Jejunum	141.79±71.00	117.08±23.40	129.13±39.42	0.65	10.35
Ileum	88.61±2.84	92.64±16.18	85.83±16.40	0.64	2.84
Villous height/crypt depth ratio					
Duodenum	5.40±1.32	4.63±1.11	4.83±0.95	0.43	0.23
Jejunum	3.16±1.45	4.63±1.07	3.58±0.90	0.07	0.28
Ileum	2.87±1.20	2.90±1.14	2.95±1.13	0.99	0.24

^{A,B,C}Means within the same row without the same superscript letter are significant different (p<0.05)

yolk weight, albumen weight, shell weight, Haugh units, albumen:yolk ratio, shell thickness, yolk color and eggshell strength (Table 4).

In accordance with Han *et al.*[6] who reported no significant effects of LPC supplementation on egg shell weight, yolk color and Haugh units. This study also did not find any effect of LPC on internal and external characteristic of egg. In exceptional, the tendency of increase of albumen weight (p = 0.06) was found when LPC was supplemented. Previous reports indicated that supplementing LPC increased egg weight by increase of albumen weight or yolk weight[2]. However, the change of albumen weight due to LPC supplementation could be explained.

Intestinal morphology: Morphology of the small intestine of hens is shown in Table 5. Supplementation of LPC significantly decrease villous height in the segment of duodenum, while the villous height in the jejunum were increased (p<0.05). The villus height:crypt depth ratio in the segment of jejunum tend to be increased when LPC was supplemented at 0.05% (p = 0.07). There were no significant effects of LPC on the other intestinal morphology observations.

The physiological functions of the gastrointestinal tract are dependent on the nutrition and quantity of feed[21] and morphology of small intestine is one of indicative biomarkers to determine gut health[22] LPC supplementation decreased duodenal villus height and increased jejunal villus height. In feed restriction studies, body weight of rabbit was declined, whereas duodenal villus height was increased[23,21]. This indicates that duodenal villus height is not always positively correlated with the growth performance of animal. Although, major absorption of nutrients occurs in the duodenum and proximal jejunum[22]. In fact duodenum is the digestive site of small intestine and the main absorptive site is jejunum. Feeding of LPC increase coefficient of total tract apparent digestibility of fatty acid in broiler diets[24,7]. Boontiam *et al.*[19] reported that LPC can function as a membrane transducer by diffusing rapidly through the lipid portion of cellular membranes to increase fluidity and permeability. Therefore, FCR of the hens fed LPC should be improved via the increase of nutrients absorption and/or transportation (jejunal morphology).

CONCLUSION

In this study, supplementation of LPC in the diet of laying hens may improve egg performance (FCR, FCE and egg size) via the enhancing of nutrient absorption (increase of villous height of Jejunum).

SIGNIFICANCE STATEMENT

This study discover the improvement of gut morphology at the segment of jejunum by the supplementation of LPC that can be beneficial for nutrient utilization (FCR) and egg production (egg size). This study will help the researcher to uncover the critical areas of gut health and phospholipids supplementation that many researchers were not able to explore. Thus a new theory on phospholipids modification, small intestinal morphology and egg production may be arrived at.

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