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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Effects of supplementation of lysophosphatidylcholine (LPC) to lying hens on production performance, fat digestibility, blood lipid profile, and gene expression related to nutrients transport in small intestine

W. Juntanapum, Ch. Bunchasak[1], T. Poeikhampha, Ch. Rakangthong and K. Poungpong

Kasetsart University, Faculty of Agriculture, Department of Animal Science
Ngam Wong Wan Rd., Lat Yao,Bangkok 10900, Thailand

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[1] Corresponding author:
e-mail: agrchb@ku.ac.th

ABSTRACT. This study was conducted to evaluate the effects of lysophosphatidylcholine (LPC) supplementation on egg production, fat digestibility, blood lipid profile and gene expression related to nutrients transport in brown egg-laying hens. In total, 384 commercial laying hens were divided into 4 groups (8 replicates, 12 animals each): 1. positive control (PC), 2. negative control (reduced energy to 75 ME kcal/kg; NC), 3. NC-LPC 0.05%, and 4. NC-LPC 0.1%, accordingly to a completely randomized design. There were no significant effects on productive performance during 33–41 weeks of age. Feed intake and feed conversion ratio (FCR) significantly increased ($P < 0.05$) in the NC group in comparison to the PC group during 42–49 weeks of age. Supplementation of LPC (at both levels) significantly reduced feed intake and FCR ($P < 0.05$). Consequently, it significantly reduced feed cost per egg weight ($P < 0.05$). In animals supplemented with LPC increased ($P < 0.05$) digestibilities of dietary fat, low-density lipoprotein cholesterol (LDL-C), triglyceride and cholesterol in blood were found. The expression of gene *BAT* in the epithelial layer of the jejunum significantly increased in the NC group, however it decreased in the NC-LPC 0.1% group ($P < 0.05$). The expression of *CAT-1* gene in the NC-LPC 0.1% group was higher than that of the PC group ($P < 0.05$). The supplemental LPC (both levels) also significantly increased the expression of the *NPC1* gene in comparison to the NC group ($P < 0.05$). So, supplementation of LPC to the diet improved the feed efficiency *via* the increase of fat digestibility and the uptake of some amino acids or cholesterol to the enterocyte up-regulating the expression of some amino acids and cholesterol transporter genes.

Introduction

Lysophosphatidylcholine (LPC) is produced from the catalytic of soy-lecithin by phospholipase A_2 activity (Joshi et al., 2006; Liu et al., 2011), so it has a greater hydrophilic properties than regular phospholipids due to the removal of one fatty acid (Joshi et al., 2006; Liu et al., 2011). LPC may be an effective natural emulsifier, since the critical micelle concentration (CMC) of LPC is 0.02–0.2 mM/l which is about 20–200 times less than bile acid (CMC = 4 mM/l) and lecithin (CMC = 0.3–2.0 mM/l) (Langmuir, 2002). Therefore, supplementing LPC seems to increase the digestion, absorption of dietary fat, and productive performance of an animal. Accordingly, Xing et al. (2004),

Zhang et al. (2011) and Jansen et al. (2015) reported that body weight and fat digestibility of piglet and broiler chicken were improved by LPC supplementation. Conversely, serum cholesterol, triglyceride was decreased (Malapure et al., 2011; Boontiam et al., 2017).

It was evidenced that LPC has an impact on forming spherical micelles in aqueous solution of the gastrointestinal (GI) tract (Vasanthakumari et al., 2011), modifying membranous proteins including ion channels (Maingret et al., 2000), and increasing the number and size of the membranous pores (Baskaran, 2003). Consequently, an increase in the flux rate of macromolecules across the cell membrane (Kelkar and Chattopadhyay, 2007; Lundbaek et al., 2010) was found due to the changing protein structure that alters the hydrophobic interface between the protein and the surrounding bilayer (Lundbaek et al., 2010). Since fat digestion, absorption, synthesis, transportation and accumulation in laying hens are important for egg production, supplementation of LPC may improve fat utilization (solubility, digestion and absorption) and/or utilization of other nutrients. Therefore, this study was conducted to evaluate the effect of LPC on productive egg performance, fat digestibility, blood lipid content and the expression of genes encoding nutrient transporters in the small intestine of laying hens.

Material and methods

Animals and management

In total, 384 Lohmann Brown-Classic laying hens were used in the 16-week experiment (33–49 weeks of age). After the 4-week adaptation period, animals were divided into 4 experimental groups accordingly to a completely randomized design. Each group consisted of 8 replications and 12 hens in each. Under an evaporative cooling system, the hens were kept in wire cages with 4 hens per cage (450 cm^2 per hen), and the lighting program was set 16 h/day. Feed and water were offered *ad libitum.*

Experimental diets

Animals were fed four experimental diets: 1. PC (positive control): ME (metabolizable energy) = 2812 kcal/kg, fat = 7.34%; 2. NC (negative control): ME = 2737 kcal/kg, fat = 5.76%; 3. NC-LPC 0.05%: ME = 2737 kcal/kg, fat = 5.76%, LPC = 0.05%; and 4. NC-LPC 0.1%: ME = 2737 kcal/kg, fat = 5.76%, LPC = 0.10% (Table 1).

LPC was sourced from Devenish Nutrition (Belfast, Northern Ireland) and marketed under the trade name Lipidol® (50% lysophosphatidylcholine along with an inert calcium silicate carrier; Easy Bio, Inc., Seoul, South Korea).

Table 1. Feed ingredients and chemical composition of experimental diets fed to animals

Indices	Group			
	positive control (PC)	negative control (NC)	NC+LPC 0.05%	NC+LPC 0.1%
Maize	54.99	57.04	57.04	57.04
Soybean 48% CP	27.36	26.99	26.99	26.99
Palm oil	4.94	3.26	3.26	3.26
DL-Methionine	0.24	0.24	0.24	0.24
L-lysine HCl	0.01	0.02	0.02	0.02
MCP 22% P	1.54	1.53	1.53	1.53
Calcium carbonate	9.72	9.72	9.72	9.72
Salt	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.40	0.40	0.40	0.40
Premixes[1]	0.50	0.50	0.50	0.50
LPC	0.00	0.00	0.05	0.10
Total	100	100	100.05	100.10
Cost/kg, bath	12.24	12.16	12.28	12.42
ME for poultry, kcal/kg	2812	2737	2737	2737
Crude protein, %	17.00	17.00	17.00	17.00
Methionine, %	0.44	0.51	0.51	0.51
Methionine+cystine, %	0.8	0.8	0.8	0.8
Lysine, %	0.9	0.9	0.9	0.9
Threonine, %	0.65	0.65	0.65	0.65
Tryptophan, %	0.19	0.19	0.19	0.19
Fibre, %	3.68	3.73	3.73	3.73
Fat, %	7.34	5.76	5.76	5.76
Calcium, %	4.10	4.10	4.10	4.10
Total P, %	0.62	0.62	0.62	0.62
Available P, %	0.42	0.42	0.42	0.42
Na, %	0.25	0.25	0.25	0.25

LPC – lysophosphatidylcholine, CP – crude protein, MCP – monocalcium phosphate; [1] contained per kg of diet, MIU: vit. A 5.0, vit. D_3 1.2; IU: vit. E 4000; g: vit. K_3 0.6, B_1 0.8, B_6 1.2, B_{12} 0.0025, nichotinic acid 5.00, pentothenic acid 3.76, folic acid 0.2, biotin 0.036, Mn 24.00, Zn 20.00, Fe 16.00, Cu 4.00, Iodine 0.8, Co 0.08, Se 0.04; and carrier added to 1 kg premix; ME – metabolizable energy

Egg production

The productive performance was divided into 2 periods (33–41 and 42–49 weeks of age, respectively). Hens were weighed at the beginning (week 33) and at the end of the study (week 49). The percentage of egg production and egg weight were recorded daily. Feed intake was measured every 14 days and reported cumulatively. From these data, egg weights, egg masses, feed intake, feed conversion ratio (FCR), feed cost per 1 kg egg (FCE) were cumulatively calculated.

Blood lipid profile

At the end of the experiment, after 6 h of feeding withdrawal, blood was randomly collected from the wing vein (one hen from each replication).

The samples were immediately transferred into non-heparinized vacuum tubes, placed at room temperature for 2 h for serum separation, and centrifuged (3000 *g*) at 4c for 10 min. The serum was removed into vials and immediately delivered to the laboratory. Consequently, the samples were stored at −20 °C until further analysed.

Total cholesterol (TC) and triglyceride (TG) concentrations in the serum were analysed with the Olympus AU400 analyser (E for L Aim Public Company Limited, Tokyo, Japan). High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were evaluated with the ABX Pentra 400 analyser (HORIBA Limited, Bangkok, Thailand).

Fat digestibility

At 49 weeks of age, the faeces from 4 hens from each replicate (32 hens/group) were randomly collected in order to evaluate the fat digestibility. During the collection period, faeces were cleaned from contaminations of scales and feathers, then immediately stored frozen at −20 °C. All representative samples were dried in an oven using air-force drying for 72 h at 60 °C. Analyses of experimental diets and dried excreta were performed using the standard protocols of AOAC International (2000) for measuring digestibility of ether extract (EE) by Soxhlet analysis and dry matter (DM). The analysed values of ingested and excreted nutrients were used to calculate apparent total tract digestibility (Khan et al., 2003):

fat digestibility (%) = [(fat intake − fat in faeces)/ fat intake] × 100.

RNA isolation and quantitative Real-Time PCR

One hen per replication (from 6 replications) was randomly selected from each pen. The small intestine (jejunum) was removed, slit open and carefully rinsed in normal saline. Mucosal enterocyte scraping by glass cover slip was used for the isolation of intestinal RNA. The samples were placed in RNAlater® solution (Ambion, Austin, TX, USA) and stored at 4 °C for 24 h prior to storage at −80 °C. Frozen tissue was disrupted in Trizol reagent (Invitrogen) to isolate total RNA. The quality of RNA was assessed using NanoDrop. One microgram of total RNA was reverse-transcribed with SuperScript II (Invitrogen, Carlsbad, CA, USA) to cDNA. Real-Time PCR consisted of total reaction volumes of 10 µl, containing 2 µl SYBR® Green Master Mixes (Applied Biosystems, Foster City, CA, USA), 2 µl (500 ng) cDNA, 0.25 µl (10 µm) forward primer, 0.25 µl (10 µm) reverse primer and 5.5 µl sterile deionized water. Quantitative PCR was performed in triplicate on the CFX Connect Real-Time System (BIO-RAD, Hercules, CA, USA) under the following conditions: an initial activation at 95° C for 15 min and followed by 40 cycles of denaturation at 94 °C for 30 s, annealing (Table 2) for 20 s and elongation at 72 °C for 30 s. Primer sequences for target transcripts were

Table 2. Primer used for real-time PCR

Gene	GenBank ID	Description or gene function	Primer sequence	Annealing temperature, °C	Reference
SGLT1	NM_001293240.1	Na^+-dependent glucose and galactose transporter	5'-CGAGATGCTGTCACTGGAGATC 3'-ACCAGTACCACAGAGTAAGGATGCT	59	Awad et al. (2014)
GLUT2	NM_207178.1	Na^+-independent glucose, galactose and fructose transporter	5'-GAGGAAACTGTGACCCGATGA 3'-ACTCTCTTTTCACTCGCAGCTTCT	57	Awad et al. (2014)
BAT	NM_001199133.1	Na^+-independent cationic and zwitterionic amino acid transporter	5'-CAGTAGTGAATTCTCTGAGTGTGAAGCT 3'-GCAATGATTGCCACAACTACCA	58	Gilbert et al. (2007)
ASCT1	XM_001232899.5	Na^+-dependent neutral amino acid transporter	5'-TTGGCCGGGAAGGAGAAG 3'-AGACCATAGTTGCCTCATTGAAT	59	Su et al. (2015)
CAT-1	NM_001145490.1	Na^+-independent cationic amino acid transporter	5'-CAAGAGGAAAACTCCAGTAATTGCA 3'-AAGTCGAAGAGGAAGGCCATAA	59	Gilbert et al. (2007)
LPL	NM_205282.1	Lipoprotein lipase	5'-GACAGCTTGGCACAGTGCAA 3'-CACCCATGGATCACCACAAA	59	Saneyasu et al. (2015)
UPC3	NM_204107.1	Avian uncoupling protein 3	5'-ACTCTGTGAAGCAGCTCTACACC 3'-ATGTACCGCGTCTTCACCACATC	59	Abe et al. (2006)
FAT/CD36	NM_001030731.1	Fatty acid translocase	5'-TGCGCTTCTTCTCCTCTGACA 3'-TCACGGTCTTACTGGTCTGGTAAAC	59	Saneyasu et al.(2015)
PLA2	NM_001277914.1	Phospholipase A2	5'-ATGAGCAGAGCTGGTGCAAA 3'-GCGGTAGGACACGTTGTAGG	59	Karray et al. (2014)
NPC1	XM_419162.6	Niemann-Pick C1/ intracellular cholesterol transporter 1	5'-CATTTTCTGCGGAACGGAGC 3'-GTGCTGACATCACTCCTGCT	59	-
GAPDH	NM_204305	Glyceraldehyde 3-phosphate dehydrogenase	5'-CCATCACAGCCACACAGAAGAC 3'-TGGACGCTGGGATGATGTT	59	Awad et al. (2014)

selected as previous reports or designed with Vector NTI (Thermo Fisher Scientific, Walthom, MA, USA) (Table 2).Relative mRNA expression was analysed by using chicken *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) mRNA expression as a reference gene. Therefore, the gene expression rates were quantitated by normalization for transcription rates of the housekeeping gene GA*PDH*. The delta-delta Ct equation was utilized to determine the relative fold-change in mRNA abundance (Livak and Schmittgen, 2001).

Statistical analyses

Data were analysed using two-way analysis of variance (ANOVA) 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. All other analyses were performed using SAS Version 9.0 (SAS, 2014), SAS Institute, Inc., Cary, NC (USA).

Results and discussion

Productive performance. Productive performances of the hens are presented in Table 3. There were no significant effects of the experimental diets during 33–41 weeks of age. However, during 42–49 weeks of age, feed intake and energy intake were significantly increased in the NC group, resulting in poor FCR ($P < 0.05$) and high FCE in comparison to the PC group. Feeding NC-LPC 0.05% and 0.1% diets significantly improved FCR and reduced FCE of the laying hens were observed ($P < 0.05$).

It was shown that during 33–41 weeks of age, a reduction in the energy content of the diet to 75 kcal has no significant negative effects on the hen productive performance. This may be connected with the fact that hens used their body energy reserves to support the energy requirement for egg production. Nevertheless, a longer period of energy depletion (42–49 weeks of age) negatively affected FCR – increased feed intake compensated energy requirement. Supplementation of LPC significantly decreased feed intake, and significantly improved the FCR in comparison to PC and NC groups. This means that LPC may improve energy utilization in an animal fed a low-energy diet by increasing the fat digestibility or absorption, or other nutrients uptake, as LPC supplementation increases the emulsification of fat or changes the structure of the lipid bilayer of the cell membrane. This is agreement with the results of Beemster et al. (2002), Attia et al. (2009), Han et al. (2010b), Boontiam et al. (2017) and Mandalawi et al. (2015) who observed improved productive performance and feed efficiency contributed to various phospholipid functions (physiological processes of the reproductive system) by supplementing LPC.

Fat digestibility and blood lipids content. Effects of LPC supplementation on fat digestibility and blood lipids content in the laying hens at 49 weeks of age are presented in Table 4. The fat digestibility in animals from the NC group was significantly lower than that from the PC group ($P < 0.05$), while in animals from the NC-LPC 0.05 and 0.1% groups fat digestibility was clearly increased ($P < 0.05$). However, the supplementation of LPC (0.05 and 0.1%)

Table 3. Effect of lysophosphatidylcholine (LPC) supplementation on egg production

Indices	Positive control (PC)	Negative control (NC)	NC + LPC 0.05%	NC + LPC 0.1%	*P*-value
Weeks 33–41					
egg production, %	95.12 ± 1.39	93.37 ± 3.66	95.01 ± 3.26	94.15 ± 3.38	>0.05
egg weight, g	65.58 ± 0.77	66.68 ± 2.88	67.26 ± 1.79	67.43 ± 1.15	>0.05
egg mass, g/hen/day	62.46 ± 1.68	62.50 ± 2.91	63.79 ± 3.50	63.28 ± 2.92	>0.05
feed intake, g/hen/day	117 ± 3.59	124 ± 5.90	120 ± 2.06	120 ± 0.89	>0.05
energy intake, kcal/kg	329 ± 10.09	339 ± 16.14	329 ± 5.62	329 ± 2.44	>0.05
FCR	1.88 ± 0.05	1.98 ± 0.03	1.89 ± 0.11	1.90 ± 0.08	>0.05
FCE, feed cost/kg egg	23.54 ± 0.60	24.10 ± 0.32	23.19 ± 1.37	23.60 ± 0.97	>0.05
Weeks 42–49					
egg production, %	91.19 ± 5.01	90.03 ± 2.98	92.78 ± 5.36	96.46 ± 0.57	>0.05
egg weight, g	68.31 ± 0.66	67.81 ± 1.69	68.54 ± 1.30	67.47 ± 2.43	>0.05
egg mass, g/hen/day	62.35 ± 3.08	60.86 ± 3.75	63.36 ± 4.04	64.90 ± 2.13	>0.05
feed intake, g/hen/day	116.10 ± 3.74^{B}	125.83 ± 3.65^{A}	118.17 ± 2.41^{B}	117.84 ± 2.11^{B}	<0.05
energy intake, kcal/kg	326.47 ± 10.5^{B}	344.38 ± 10.00^{A}	323.43 ± 6.61^{B}	322.52 ± 5.79^{B}	<0.01
FCR	1.87 ± 0.13^{B}	2.07 ± 0.07^{A}	1.87 ± 0.12^{B}	1.82 ± 0.04^{B}	<0.05
FCE, feed cost/kg egg	23.43 ± 1.65^{AB}	25.18 ± 0.83^{A}	22.97 ± 1.42^{B}	22.53 ± 0.51^{B}	<0.05

FCE – feed cost per 1 kg egg; FCR – feed conversion ratio (feed intake/egg mass); values are express as mean ± standard deviation (SD); means were obtained from 8 replicates (12 birds each); ^{ABC} – means within the same row without the same superscript are significantly different at $P < 0.05$

Table 4. Effect of lysophosphatidylcholine (LPC) supplementation on blood lipid level and fat digestibility in laying hens

Indices	Positive Control (PC)	Negative Control (NC)	NC+LPC 0.05%	NC+LPC 0.1%	*P*-value
Fat digestibility*,%	95.29 ± 0.87^{A}	91.59 ± 0.79^{B}	95.13 ± 0.47^{A}	95.18 ± 0.68^{A}	<0.01
Cholesterol**, mg/dl	86.80 ± 18.47^{AB}	57.25 ± 15.73^{B}	95.43 ± 31.39^{A}	100.68 ± 29.45^{A}	<0.05
Triglyceride**, mg/dl	521.80 ± 101.92^{AB}	424.00 ± 132.69^{B}	642.30 ± 158.55^{A}	654.78 ± 128.11^{A}	<0.05
LDL-C**, mg/dl	18.80 ± 4.78^{AB}	14.67 ± 4.48^{B}	26.00 ± 6.36^{A}	25.33 ± 7.94^{A}	<0.01
HDL-C**, mg/dl	36.00 ± 4.80	37.75 ± 4.03	40.58 ± 5.74	38.17 ± 14.47	>0.05

LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; the values are expressed as mean ± standard deviation (SD); * – calculated from 8 hens/treatment group; ** – calculated from 8 replicates (4 hens per replicate)/treatment group; ^{ABC} – means within the same row without the same superscript are significantly different at $P < 0.05$

significantly increased triglyceride, cholesterol, and LDL-C in the blood in compariosn to the NC group ($P < 0.05$).

It is known that the supplementation of LPC to the low-energy diet increased the digestibility of dietary fat. Accordingly, Zhao et al. (2015) found that weaning pigs fed a restricted energy diet with the inclusion of LPC at the level of 0.05% had greater digestibility of fat. In broiler chickens, high digestibility of dietary fat and fatty acids as a result of LPC supplementation was also reported (Han et al., 2010a; Jansen et al., 2015; Allahyari-Bake and Jahanian, 2017). Since LPC has more hydrophilic property than regular phospholipids and bile acid (Langmuir, 2002; Joshi et al., 2006; Liu et al., 2011), supplementing of LPC would increase the emulsification and digestibility of fat. Another reason is that LPC is a part of the phospholipid bilayers and acts as an important regulator in modifying fluidity, it would also improve permeability of the lipid bilayer (Shumilina et al., 2006). Therefore, the increment of fat digestibility and blood lipids content in this study confirms the increase in fat emulsification and/or modification of the lipid bilayer of the enterocyte of laying hens.

Gene expression. Effects of LPC supplementation into diet on the expression of genes related to nutrients transport in the small intestine at 49 weeks of age are presented on Figures 1, 2 and 3. The expression of gene related to the Na+-independent cationic and zwitterionic amino acid transporter (*BAT)* was significantly increased in the NC group ($P < 0.05$), while supplemental LPC downregulated the expression of this gene to the values observed for the PC group. Conversely, *CAT-1* expression in the NC-LPC 0.1% group was significantly higher than that in the PC group ($P < 0.05$). The expression of the *NPC1* gene (Niemann-Pick C1/intracellular cholesterol transporter 1) that is involved in the cholesterol uptake was significantly higher in the NC-LPC 0.05 and 0.1% groups in comparison to the NC group ($P < 0.05$).

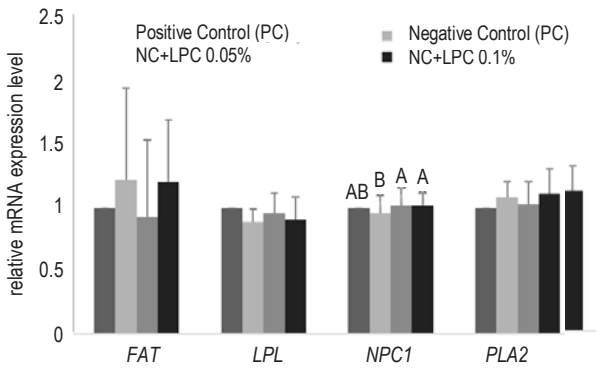

Figure 1. Expression of genes related to fat transport in jejunum: *FAT*, *LPL*, *NPC1*, *PLA2*. Bars present means ± standard deviation (SD), bars with different superscripts are significantly different at $P < 0.05$

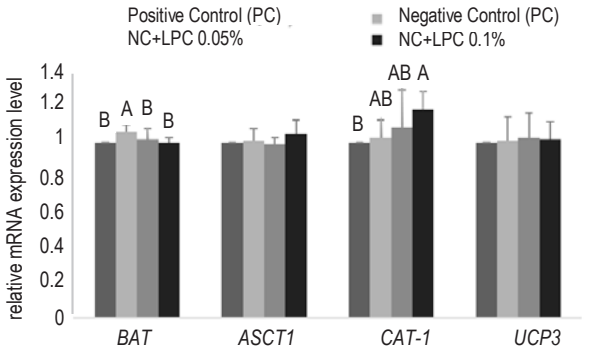

Figure 2. Expression of genes related to amino acids transport in jejunum: *BAT*, *ASCT1*, *CAT-1* and *UCP3*. Bars present means ± standard deviation (SD), bars with different superscripts are significantly different at $P < 0.05$

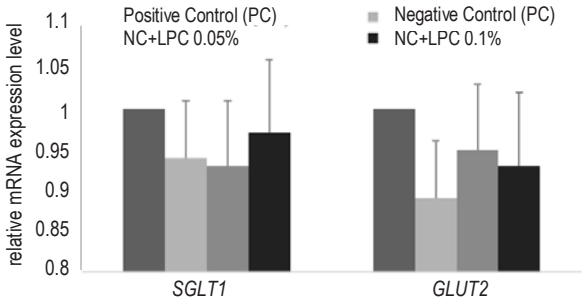

Figure 3. Expression of genes related to glucose transport in jejunum: *SGLT1* and *GLUT2*. Bars present means ± standard deviation (SD)

Although gene expression processes are genetically preprogramed, dietary nutrients can activate these processes (Liao et al., 2015; Zhou et al., 2018). The regulation of nutrient transporters in the small intestine is connected with the nutrients present in the diet (Gilbert et al., 2008). Amino acids are transported by neutral, acidic or basic amino acids transporters (Wu, 2013). The *BAT* gene is encoding the major apical neutral amino acid transporter in the kidney and the small intestine (Bröer, 2008). Although system BAT has broad substrate specificity, the branched chain (leucine, isoleucine) and benzenoid amino acids (tryptophan and phenylalanine) are also preferred (Van Winkle et al., 2006). A reduction of dietary energy content significantly increased the expression of the *BAT* gene. This may be due to the adaptive regulation linking substrate availability to amino acid transport (Gazzola et al., 1972). Hatzoglou et al. (2004) illustrated the increased transport activity in cells exposed to limited substrate, called adaptive depression. Branched chain amino acids (BCAAs) are essential amino acids, and also regulate many key signalling pathways (Zhang et al., 2017), including secretion of insulin (Nair and Short, 2005), fat metabolism, glucose metabolism, glucose transportation, intestinal barrier function and absorption (Zhang et al., 2017). Therefore, the decrease of energy in the diet may enhance the expression of the *BAT* to compensate the energy requirementation, while supplementation of LPC to a low-energy diet down-regulates this gene expression due to the increasing fat utilization.

CAT-1 is the major system y+transporter in most cells (system y+, namely its ability to recognize cationic amino acids; lysine and arginine), so it supports the important metabolic functions such as synthesis of proteins, nitric oxide (NO), polyamine and interorgan amino acid flow (Hatzoglou et al., 2004). Lysine is the second limiting amino acid in poultry, and it is generally known that lysine improves productive performance in hens. Different dietary standardised ileal digestible Lys levels affecting the mRNA level of *CAT1* in jejunum in weaned pig have been reported (García-Villalobos et al., 2012; Wang et al., 2012). Therefore, it may be hypothesized that the increase of *CAT-1* gene expression may increase the lysine uptake to promote egg performance by LPC supplementation, since this improvement has to be supported by both dietary energy and amino acids.

Niemann-Pick C1-Like 1 (NPC1) is localized in the apical membrane of the small intestine absorptive enterocytes that is essential for intestinal sterol absorption (Betters et al., 2010). Thus, NPC1 plays an essential role in the intestinal cholesterol absorption (Altmann et al., 2004). Cholesterol is precursor of sterol hormone, so high level cholesterol is needed by hen to accumulate in the egg, and also for proper function of the hypothalamo-pituitary-ovarian axis and whole reproductive system. Lack of NPC1 reduces weight gain in animals fed a diabetogenic diet (Eric et al., 2008). In the present study, therefore, it is not surprising that the increase in blood cholesterol is paralleled by an increase in the expression of the *NPC1* gene. Accordingly, the fat digestibility of laying hens fed low-energy diet supplemented with LPC was improved. This indicates that LPC not only increases the emulsification of fat (increased fat digestibility), but also increases the uptake of cholesterol from the small intestine *via* the enhancement of the *NPC1* gene expression. Consequently, the FCR and FCE in hens fed LPC-supplemented diets were better than in those from PC and NC groups.

Conclusions

Supplementing lysophosphatidylcholine (LPC) at the levels of 0.05 and 0.1% into a low-energy diet improves feed conversion ratio and feed cost per 1 kg egg in laying hens due to the increment of fat digestibility and absorption. Furthermore, LPC also up-regulates the expression of *CAT-1* and *NPC1* genes to increase the uptake of some amino acids and cholesterol into the enterocyte.

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