Effects of lysophospholipid supplementation to lower nutrient diets on growth performance, intestinal morphology, and blood metabolites in broiler chickens

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ABSTRACT The purpose of this research was to investigate the effects of dietary lysophospholipid (LPL) supplementation on low-energy, crude protein, and selected amino acids on growth performance, intestinal morphology, blood metabolites, inflammatory response, and carcass traits in broiler chickens. A total of 300 one-day-old male chicks (Ross 308) were assigned to 5 treatments, with 6 replications of 10 birds each in a completely randomized design. The 5 treatments were: positive control (PC) without LPL supplementation and adequate in all nutrients, negative control (NC) without LPL, and reduced 150 kcal/kg of metabolizable energy and reduced 5 to 6% of crude protein and selected amino acids including Lys, Met, Thr, and Trp in a calculated amount relative to the PC, NC + 0.05% LPL (LPL05), NC + 0.10% LPL (LPL10), and NC + 0.15% LPL (LPL15). Feeding LPL linearly improved growth performance, feed conversion ratio, ether extract, and protein digestibility. LPL supplementation on low-energy and nitrogenous diets showed significant enhancements in metabolic profiles of blood glucose, protein utilization, and immune system functions. These improvements influenced carcass composition, especially in relative weights of pancreas and breast muscle. In contrast, LPL addition showed no significant effects on relative weights of immune organs, gizzard, and abdominal fat. The NC birds were more susceptible to inflammation via modulating the secretion of interleukin-1 (IL-1) and increasing crypt depth in the jejunal and duodenal segments. However, the inclusion of 0.05% LPL to the NC diet could alleviate inflammation with increased jejunal villi height, ratio of villi height to crypt depth, and decreased IL-1 level. Overall, LPL promotes growth performance, nutrient utilization, gut health, anti-inflammation, and muscle yields when applied to diets of broiler chickens with lower levels of energy, crude protein, and selected amino acids.

Key words: lysophospholipid, lower nutrient diets, broiler chicken, growth performance, intestinal morphology

2016 Poultry Science 00:1–9
http://dx.doi.org/10.3382/ps/pew269

INTRODUCTION

Fast-growing broilers require energy and nitrogenous compounds for supporting growth and performance. However, there are still controversial issues on feeding young birds high amounts of nutrients (Widyaratne and Drew, 2011; Rochell et al., 2016). Emulsifiers play an important role in aiding micelle formation. Lysophospholipids (**LPLs**) are natural surfactants of hydrolyzed soy lecithin, which are produced by phospholipase A_2 to cleave one hydrophobic fatty acid from phospholipids (Joshi et al., 2006). Thus they are more efficient than lecithin in emulsifying properties and the subsequent effect on fat hydrolysis. The LPL's higher hydrophilic–lipophilic balance values of 2 to 12, which is higher than bile and lecithin (Van Nieuwenhuyzen and Tomás, 2008), and lower critical micelle concentration (0.02 to 0.2 mM/L) make it more effective than modified lecithin. This indicates the ability to form smaller micelles in the guts of animals and cause larger surface areas of lipid droplets for pancreatic lipases to interact. LPL also alters protein channel formation in the membrane by increasing ion exchanges (Maingret et al., 2000). Change in deformation energy increased the number and size of the membranous pores and consequently increased the flux rate of macromolecules across the cell membrane (Kelkar and Chattopadhyay, 2007; Lundbæk et al., 2010). Both mechanisms induce the transport of nutrients, from small particles such as calcium ions to large components such as polysaccharides to be broken down for absorption, leading to higher nutrient bioavailability for promising broiler performance.

Besides the important role in increasing membrane permeability for better lymphatic absorption of lipophilic substance (Nakano et al., 2009), the derivative lecithins also act as immunostimulants by promoting the influx of monocytes and enhancing macrophages

Received April 11, 2016.
Accepted June 20, 2016.
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during the pathogenic invasion (Ousman and David, 2000). This was consistent with Lewis et al. (2016), who demonstrated the activation of lipid-soluble phosphatidylcholine in modulating cell proliferation and interleukin-2 secretion, and consequently promoted cell-mediated immunity. Lecithin derivatives also have been shown to prevent cellular damage (Maingret et al., 2000; Skoura and Hla, 2009) and improve broiler performance through increasing nutrient utilization (Raju et al., 2011; Jansen et al., 2015). Based on these findings, we hypothesized that LPL would be a good material for improving nutrient digestion and absorption as well as activating the immune system of broiler chickens. However, supplementation of new biosurfactant LPL is not well known in broiler chicken studies, especially when applied in diets lower in ME, CP, and amino acids (**AA**). Consequently, the aim of the current study was to investigate the effects of dietary LPL supplementation in diets with lower ME, CP, and AA on growth performance, nutrient digestibility, intestinal morphology, and blood profiles in broiler chickens.

MATERIALS AND METHODS

All procedures for bird care and handling were approved by the national research committee of Animal Ethics, Seoul National University (Gwanak, South Korea).

Experimental Design, Diets, and Management

A total of 300 one-day-old Ross 308 male chicks were obtained from a local hatchery (Busung Farms, South Korea). The chicks were individually weighed on an electronic scale (model PB 1501, Mettler, Toledo, OH) and assigned to pens with 10 chicks of 6 replications using a completely randomized design. The pen size was a 1.42 m × 1.31 m (1.86 m^2/pen) providing a stocking density of 5.38 birds/m^2 (10 birds/pen). Each pen was equipped with tube feeders, automatic waterers, and rice hall. The five treatments were: positive control (**PC**) formulated with adequate amounts of ME, CP, and AA with no supplementation of LPL, negative control (**NC**) without LPL supplementation, and reductions of 150 ME kcal/kg and 5 to 6% of CP and selected essential AA including Lys, Met, Thr, and Trp in PC diet, NC + 0.05% LPL (**LPL05**), NC + 0.10% LPL (**LPL10**), and NC + 0.15% LPL (**LPL15**). The LPL derived from soy lecithin was obtained from Easy Bio Inc., (LIPIDOL, Seoul, South Korea). The experimental diets during starter (d zero to 7), grower (d 8 to 21), and finisher (d 22 to 35) periods were formulated according to the nutrient requirements for Ross 308 broilers (Aviagen, 2007; Table 1). Broilers were raised on rice hull in a temperature-controlled environment for a 35 d feeding. The temperature was controlled at 34 ± 2°C for the first 3 d using a heat brooder, and gradually declined thereafter to 22 to 24°C. During the course of the experiment, a lighting program offered a 23-h photoperiod (23L:1D) according to the management guidelines of commercial broilers. Drinking water and feed were provided ad libitum throughout the study.

Performance Measurements

Broiler BW and feed intake (**FI**) were recorded on pen basis at the d of hatch, 7, 21, and 35 days. The data were used to calculate body weight gain (**BWG**), FI, and feed conversion ratio (**FCR**) in each period and cumulatively. Mortality was recorded daily to adjust FI.

Nutrient Digestibility

The metabolic trial was conducted during 17 to 23 d of age. Experimental diets for the adjustment period were given for 4 d, and total collection of residue feed was collected from d 21 to 23. During the collection period, contaminations of scales, feather, and filoplumes were removed from the fecal samples every 12 h, then immediately stored frozen at −20°C. All representative samples were pooled and dried in an oven using air-force drying for 72 h at 60°C. Experimental diet and dried excreta analyses were performed using the standard protocols of AOAC International (2000) for measuring nutrient digestibility of CP by the Kjeldahl procedure (method 984.13), ether extract (**EE**) by the Soxhlet analysis (method 920.39), and DM (method 930.15). The analyzed values of ingested and excreted nutrients were used to calculate apparent total tract digestibility. Each sample was run in triplication.

Intestinal Morphology

Tissue samples of 30 birds were collected at the end of the experiment. The samples from the duodenum and jejunum were rinsed with buffered saline solution. An approximately 5 cm piece of the middle part of each segment was excised and fixed in 10% (vol/vol) saline solution for histomorphological measurements. Tissues from the segments preserved in 10% neutral formaldehyde solution were cut into 10 cross sections (approximately 3 μm thickness) and were fixed on slides for staining with hematoxylin and eosin. Villous height was defined from the villous tip to the villus to crypt junction, whereas crypt depth was measured from the villous bottom to the crypt. The ratio of villous height and crypt depth (**VH:CH ratio**) was examined. Morphological studies were performed on a light microscope (Olympus Corporation, Tokyo, Japan) with stereological software analysis using Version 2.3.1.3 (Visiopharm Albertslund, Hørsholm, Denmark).

Blood Collection and Analyses

Blood was randomly collected from jugular veins after a 2-hr feeding withdrawal from 6 birds at the

Table 1. Composition and nutrient specifications for the experimental diets (%, as-fed basis).

	Starter (d 0 to 7)		Grower (d 8 to 21)		Finisher (d 22 to 35)	
Item	PC	NC	PC	NC	PC	NC
Corn						
Fine particle	40.81	44.61	28.16	30.60	15.27	16.40
Coarse particle	13.60	14.87	28.15	30.60	45.82	49.22
Soybean meal	30.98	27.93	27.62	24.68	22.81	20.13
DDGS	3.00	3.00	3.00	3.00	3.00	3.00
Meat meal	3.00	3.00	4.00	4.00	4.00	4.00
Soybean oil	3.73	1.00	5.20	2.50	5.44	2.80
L-lysine sulfate (55%)	0.45	0.47	0.25	0.28	0.23	0.25
DL-methionine (98%)	0.37	0.34	0.29	0.27	0.25	0.23
Threonine (98%)	0.13	0.13	0.07	0.06	0.05	0.06
Tryptophan	0.03	0.07	0.00	0.04	0.00	0.04
Choline	0.08	0.08	0.07	0.07	0.08	0.08
Dicalcium phosphate	2.10	2.10	1.70	1.70	1.60	1.60
Limestone	1.11	1.79	0.89	1.60	0.85	1.59
Dried salt	0.30	0.30	0.30	0.30	0.30	0.30
$NaHCO_3$	0.06	0.06	0.05	0.05	0.05	0.05
Vitamin premix[1]	0.13	0.13	0.13	0.13	0.13	0.13
Mineral premix[2]	0.12	0.12	0.12	0.12	0.12	0.12
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated value[3]						
ME (kcal/kg)	3,025	2,875	3,150	3,000	3,200	3,050
CP (%)	22.00	20.90	21.00	19.95	19.00	18.05
Ca (%)	1.05	1.29	0.90	1.15	0.85	1.12
Available P (%)	0.51	0.51	0.45	0.45	0.43	0.43
Lysine (%)	1.40	1.34	1.23	1.17	1.09	1.03
Methionine (%)	0.69	0.65	0.60	0.57	0.54	0.51
Threonine (%)	0.94	0.90	0.84	0.80	0.75	0.72
Tryptophan (%)	0.25	0.23	0.23	0.22	0.20	0.19

PC = positive control, NC = negative control, DDGS = distiller's dried grains with solubles.

[1]Provided the following quantities of vitamin mixture per kilogram of complete diet: vitamin A (retinyl acetate), 11,000 IU; vitamin D_3, 5,000 IU; vitamin E (dl-α-tocopheryl acetate), 60 mg; vitamin K, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 8 mg; vitamin B_6, 4 mg; vitamin B_{12}, 16 μg; niacin, 60 mg; folic acid, 2 mg; biotin, 130 μg; and calcium pantothenic acid, 20 mg.

[2]Provided the following quantities of mineral mixture per kilogram of complete diet: Cu (copper sulfate), 29 mg; Zn (zinc sulfate), 108 mg; Mn (manganese sulfate), 115 mg; Fe (ferrous sulfate), 60 mg; and Se (sodium selenite), 0.4 mg.

[3]Calculated values as-fed basis.

beginning of the experiments and 30 birds at the end of the experiments (one sample from each replication). The samples were immediately transferred into non-heparinized vacuum tubes, placed at room temperature for 2 h for serum separation, and thereafter centrifuged (3,000 × g) at 4°C for 10 minutes. The serum without supernatant was removed into vials and immediately delivered to the laboratory for further biochemical analyses. Serum interleukin-1 (IL-1, catalog no. CSB-E10069Ch), interleukin-6 (IL-6, catalog no. CSB-E08549Ch), and tumor necrosis factor alpha (TNF-α, catalog no. CSB-E11231Ch) were analyzed using commercial ELISA kits (Cusabio Biotech Inc., Wuhan, China). Circulating serum glucose (catalog no. 11876899 216) was measured using the enzymatic kinetic method (Glucose Hexokinase Kit, Roche Dianostics, Indianapolis, IN), and serum free fatty acid (FFA, catalog no. 438–91691) was determined using commercial kits (Wako Pure Chemical Industries, Osaka, Japan). Uric acid concentration (catalog no. P803-OU982-01) was quantified using commercial reagent kits (Pointe Scientific, Canton, MI). All representative samples were performed twice and measured immediately to avoid variations.

Carcass and Intestinal Organ Measurements

After collecting the blood, the selected birds were sacrificed by cervical dislocation for carcass measurements. Carcass components of immune organs (thymus, spleen, and bursa of Fabricius), gizzard, pancreas, breast, leg muscle, and abdominal fat were weighed on an electronic scale for further calculation of relative carcass weights.

Statistical Analysis

Data were analyzed in a completely randomized design using the GLM procedure of SAS software (SAS Institute, Cary, NC). The pen was an experimental unit for growth performance, whereas all selected birds from each replication were defined as the experimental unit for nutrient digestibility, blood metabolite, morphological, and carcass measurements. Significant differences among treatments were separated by Duncan's new multiple range test at the probability of $P < 0.05$ and $P < 0.01$. All criteria were assessed for linear and quadratic effects of LPL supplementation by

orthogonal polynomial contrast. The significant difference for a tendency was defined as $P > 0.05$ to $P < 0.10$.

RESULTS

Growth Performance

As shown in Table 2, there were no significant differences in BW, BWG, FI, and FCR in the starter period. The significant effects for BW and BWG were observed during the growing ($P < 0.05$) and finishing phases ($P < 0.01$), respectively, compared to NC. There was a linear increase of bird BW in the grower period ($P < 0.001$), and both linear and quadratic effects during the finisher period ($P < 0.001$), with an increasing LPL level. Body weight gain tended to increase from 789.35 to 840.51 g/bird during the grower period ($P = 0.066$) with the increasing LPL level. The linear and quadratic improvements for BWG with supplementation of LPL also were observed both in the finishing and overall periods ($P < 0.001$). Furthermore, the LPL could stimulate the FI of birds in the finisher and overall periods, respectively (linear and quadratic effects; $P < 0.001$). Feed conversion ratio decreased linearly ($P = 0.003$) and quadratically ($P = 0.007$) when the LPL supplementation level was increased in the finisher phase. Diets supplemented with LPL significantly improved growth performance during the entire period, as compared to NC treatment ($P < 0.01$), but there were no differences between the PC and the LPL groups.

Nutrient Digestibility

Nutrient digestibility for DM, CP, and EE are summarized in Table 3. The dietary treatments had no considerable difference in DM digestibility. However, the linear and quadratic effects for digestibility of CP ($P = 0.002$ and $P = 0.014$, respectively) and the linear effect for EE ($P = 0.025$) were detected as the LPL level increased. Furthermore, supplementation of LPL showed an improvement in digestibility of CP ($P < 0.01$) and EE ($P < 0.05$) over those of NC birds.

Table 2. Effects of LPL supplementation to lower nutrient diets on growth performance in broiler chickens.[1,2]

							P-value	
Criteria	PC	NC	LPL05	LPL10	LPL15	SEM	Linear	Quadratic
BW (g/bird)								
1 wk	182.45	180.32	179.63	182.53	182.49	2.980	0.618	0.932
3 wk	1,038.83^{a}	969.67^{b}	977.45^{b}	1,018.26^{a,b}	1,023.00^{a,b}	10.651	0.038	0.755
5 wk	2,240.37^{A}	1,649.00^{B}	2,123.07^{A}	2,121.46^{A}	2,173.63^{A}	44.153	<0.001	<0.001
BWG (g/bird)								
0 to 1 wk	140.42	138.29	137.60	140.50	140.46	2.980	0.618	0.932
2 to 3 wk	856.38^{a}	789.35^{b}	797.81^{a,b}	835.74^{a,b}	840.51^{a,b}	10.357	0.066	0.763
4 to 5 wk	1,201.54^{A}	679.33^{B}	1,145.63^{A}	1,103.21^{A}	1,150.63^{A}	39.745	<0.001	<0.001
0 to 5 wk	2,198.34^{A}	1,606.97^{B}	2,081.04^{A}	2,079.43^{A}	2,131.60^{A}	44.153	<0.001	<0.001
FI (g/bird)								
0 to 1 wk	152	151	152	152	152	2.523	0.812	0.868
2 to 3 wk	1,198	1,162	1,169	1,185	1,192	11.934	0.391	0.799
4 to 5 wk	1,951^{A}	1,395^{B}	2,001^{A}	1,967^{A}	2,011^{A}	51.435	<0.001	<0.001
0 to 5 wk	3,301^{A}	2,708^{B}	3,322^{A}	3,304^{A}	3,355^{A}	56.033	<0.001	<0.001
FCR (feed : gain)								
0 to 1 wk	1.09	1.11	1.11	1.09	1.08	0.015	0.589	0.912
2 to 3 wk	1.40	1.47	1.47	1.42	1.42	0.015	0.208	0.946
4 to 5 wk	1.63^{B}	2.06^{A}	1.75^{B}	1.79^{B}	1.75^{B}	0.035	0.003	0.007
0 to 5 wk	1.50^{B}	1.68^{A}	1.60^{A,B}	1.59^{A,B}	1.58^{B}	0.015	0.012	0.083

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL, BW= body weight; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio.

[1]All experimental birds were fed diets from the average initial BW was 42.03 g/bird to the average final weight was 2,061.51 g/bird.

[2]Values are expressed as means of 60 birds represented from each treatment (10 birds per replication, N = 30).

[a,b]Means in a same row with different superscripts significantly differ ($P < 0.05$).

[A,B]Means in a same row with different superscripts significantly differ ($P < 0.01$).

Table 3. Effects of LPL supplementation on nutrient digestibility during 21 to 23 d of age.[1]

							P-value	
Criteria	PC	NC	LPL05	LPL10	LPL15	SEM	Linear	Quadratic
Digestibility (%)								
Dry matter	63.63	64.04	64.61	63.46	64.97	1.446	0.916	0.765
Crude protein	68.40^{A,B}	66.01^{B}	74.82^{A}	74.93^{A}	74.63^{A}	1.148	0.002	0.014
Ether extract	67.36^{a,b}	64.54^{b}	80.10^{a}	79.93^{a}	80.03^{a}	2.194	0.025	0.079

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL.

[1]Values are expressed as means of 6 birds represented from each treatment and (one bird per replication, N = 30).

[a,b]Means in a same row with different superscripts significantly differ ($P < 0.05$).

[A,B]Means in a same row with different superscripts significantly differ ($P < 0.01$).

Intestinal Morphology

No linear and quadratic effects were observed with LPL inclusion in all criteria of intestinal mucosa (Table 4). Jejunal villous height was significantly increased in broiler chickens fed LPL05 as compared to those in the PC treatment ($P < 0.05$). In addition, supplementation level of LPL05 showed positive improvement in the VH:CD ratio of the jejunum ($P < 0.05$), and diminished crypt depth in the duodenum ($P < 0.01$) and jejunum ($P < 0.01$). However, these criteria had no significant effect on either linear or quadratic responses when increasing the LPL level.

Blood Metabolic Profiles

Blood metabolites of inflammatory response at the initial and the terminal periods are indicated in Table 5. There was a reducing tendency of TNF-α regardless of LPL level ($P = 0.082$). The IL-1 concentration was significantly higher in the group fed the NC diet than that of the group fed the PC diet ($P < 0.05$). No significant effect on IL-6 concentration in broilers was found among the treatments.

The metabolic profile of glucose concentration was linearly ($P = 0.003$) and quadratically ($P = 0.029$) increased as the LPL supplementation level increased. The LPL10 and LPL15 had higher glucose concentration than the NC ($P < 0.01$). Serum FFA concentration was lowered in birds fed PC compared with NC ($P < 0.05$), but there were no differences between the PC and the LPL15 treatments. In addition, the LPL15 had lower uric acid concentration compared to those fed the NC diet ($P < 0.01$). The linear reduction in uric acid concentration also was observed ($P = 0.001$) as the inclusion rate of LPL increased.

Table 4. Effects of LPL supplementation on intestinal morphology in broiler chickens at 35 d of age.[1]

							P-value	
Criteria	PC	NC	LPL05	LPL10	LPL15	SEM	Linear	Quadratic
Villous height (μm)								
Duodenum	981.42	978.73	1,076.95	1,177.46	1,092.90	33.581	0.153	0.919
Jejunum	847.56^{b}	917.28^{a,b}	1,072.95^{a}	1,025.83^{a,b}	1,045.36^{a,b}	34.474	0.315	0.245
Crypt depth (μm)								
Duodenum	193.15^{A,B}	203.51^{A}	166.29^{B}	190.64^{A,B}	193.94^{A,B}	4.037	0.913	0.074
Jejunum	121.81^{C}	158.01^{A,B}	133.16^{B,C}	161.54^{A,B}	170.41^{A}	4.761	0.114	0.375
VH:CD ratio								
Duodenum	5.10	5.05	6.57	6.20	5.65	0.246	0.554	0.374
Jejunum	7.02^{a,b}	5.86^{b}	8.13^{a}	6.41^{b}	6.25^{b}	0.276	0.837	0.090

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL.
[1]Values are expressed as means of 6 birds represented from each treatment (one bird per replication, N = 30).
[a,b]Means in a same row with different superscripts significantly differ ($P < 0.05$).
[A–C]Means in a same row with different superscripts significantly differ ($P < 0.01$).

Table 5. Effects of LPL supplementation on blood metabolites in broiler chickens at 35 d of age.[1]

							P-value	
Criteria	PC	NC	LPL05	LPL10	LPL15	SEM	Linear	Quadratic
TNF-α (pg/mL)								
Initial[2]	6,819.59	6,819.59	6,819.59	6,819.59	6,819.59	–	–	–
d 35	2,608.55	2,998.58	2,742.57	2,630.98	2,643.43	56.772	0.082	0.402
IL-1 (pg/mL)								
Initial[2]	142.18	142.18	142.18	142.18	142.18	–	–	–
d 35	65.48^{b}	90.09^{a}	80.12^{a,b}	76.67^{a,b}	76.56^{a,b}	3.019	0.117	0.408
IL-6 (pg/mL)								
Initial[2]	10.17	10.17	10.17	10.17	10.17	–	–	–
d 35	8.08	9.14	8.94	7.91	8.94	0.234	0.513	0.457
Glucose (mg/dL)								
Initial[2]	94.67	94.67	94.67	94.67	94.67	–	–	–
d 35	254.50^{A}	186.17^{C}	216.00^{B,C}	219.00^{B}	223.33^{B}	5.029	0.003	0.029
FFA (mg/dL)								
Initial[2]	151.37	151.37	151.37	151.37	151.37	–	–	–
d 35	326.50^{b}	541.33^{a}	549.83^{a}	496.33^{a}	482.17^{a,b}	28.979	0.411	0.964
Uric acid (mg/dL)								
Initial[2]	4.80	4.80	4.80	4.80	4.80	–	–	–
d 35	4.78^{A}	4.60^{A}	4.35^{A,B}	3.12^{B}	3.12^{B}	0.196	0.001	0.668

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL, TNF-α = Tumor necrosis factor alpha; IL-1 = Interleukin-1; IL-6 = Interleukin-6, FFA = free fatty acids.
[1]Values are expressed as means of 6 birds represented from each treatment (one bird per replication, N = 30).
[2]Initial value is represented as mean of 6 birds collected samples at one d of age (N = 6).
[a,b]Means in a same row with different superscripts significantly differ ($P < 0.05$).
[A–C]Means in a same row with different superscripts significantly differ ($P < 0.01$).

Table 6. Effects of LPL supplementation on relative immune organs and carcass weights in broiler chickens at 35 d of age.[1]

							P-value	
Criteria	PC	NC	LPL05	LPL10	LPL15	SEM	Linear	Quadratic
Immune organs (g/100 g BW)								
Thymus	0.46	0.44	0.47	0.45	0.44	0.014	0.936	0.786
Spleen	0.09	0.09	0.07	0.08	0.07	0.005	0.159	0.244
Bursa	0.23	0.21	0.24	0.22	0.21	0.006	0.710	0.410
Carcass traits (g/100 g BW)								
Gizzard	2.24	2.24	2.26	2.25	2.25	0.057	0.951	0.950
Pancreas	$0.27^{a,b}$	0.23^{b}	0.29^{a}	0.29^{a}	0.29^{a}	0.008	0.035	0.119
Breast	$18.63^{A,B}$	15.71^{B}	18.96^{A}	$18.36^{A,B}$	20.19^{A}	0.405	0.002	0.005
Leg muscle	$18.12^{a,b}$	17.50^{b}	18.79^{a}	$18.36^{a,b}$	$18.48^{a,b}$	0.157	0.100	0.044
Abdominal fat	1.13	1.21	1.17	1.19	1.18	0.064	0.936	0.859

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL.
[1]Values are expressed as means of 6 birds represented from each treatment (one bird per replication, N = 30).
[a,b]Means in a same row with different superscripts significantly differ ($P < 0.05$).
[A,B]Means in a same row with different superscripts significantly differ ($P < 0.01$).

Relative Weights of Immune Organs and Carcass Components

The effects of LPL supplementation to NC diet on relative weights of immune organs and carcass weights in broiler chickens are summarized in Table 6. The relative weight of the breast showed the linear and quadratic effects ($P = 0.002$ and $P = 0.005$, respectively), whereas only the linear effect was observed for the relative weight of the pancreas ($P = 0.035$). No significant effects were observed on the relative weights of thymus, spleen, bursa of Fabricius, gizzard, and abdominal fat pad among dietary treatments. However, feeding LPL15 had a significant effect on the relative weights of breast ($P < 0.01$) in comparison to those fed NC. In addition, the weight of the leg muscle was lowered in the NC-supplemented birds compared with LPL05-supplemented birds ($P < 0.05$).

DISCUSSION

Growth Performance

The use of LPL is important for commercial broilers under the limitations of energy and nitrogenous diets. The current research found LPL supplementation beneficial for adequate nutrient supply as well as activation of various functions in the body. Some studies showed improvement in body weight gain (Emmert et al., 1996) and G:F ratio (Khonyoung et al., 2015) of young birds fed emulsifiers. The previous results are in agreement with our findings, regardless of BW and BWG. This supports the important roles of phospholipids in fat digestion by their emulsifying properties and nutrient absorption via increasing micelle formations (Schwarzer and Adams, 1996), resulting in better growth performance in young chicks.

Nutrient Digestibility

In low nutrient diets, LPL has been proven to increase the nutrient digestibility and energy values in feed. According to Zhao et al. (2015), who observed that weaning pigs fed a restricted energy diet at 0.30 MJ/kg in early and late weaning periods with the inclusion level of LPL at 0.05% had greater digestibility of DM, gross energy, CP, and EE than the basal and reduced nutrient diets without LPL supplementation, which was in accordance with the current findings. The fatty acids, EE, and AME_n values were improved by feeding phospholipids to broilers (Jansen et al., 2015). In the current experiment, the digestibility of CP and EE in LPL treatments was greater than those in NC. These results agreed with the observations of Han et al. (2010), who showed that addition of lysolecithin at 0.10% in laying hen diets could maximize digestibility of nitrogen, energy, and AA. Modified lecithins are known to be an integral part of phospholipid bilayers (Shumilina et al., 2006). They act as important regulators in modifying fluidity and permeability of lipid bilayers by decreasing deformation energy, which directly affects the stability of the cell membrane. This means that the coupling between integral membrane proteins and their surrounding lipid bilayers will alter the hydrophobic interface to enter the protein channel (Lundbæk et al., 2010). This process causes an increased flux rate of various nutrients as well as promoted absorption of lipid and lipophilic substances for entering the enterocyte (Cohn et al., 2010). Once it is taken up by the enterocyte, the LPL is converted to phospholipids and the delivery of absorbed lipids is further assisted by chylomicrons (Nakano et al., 2009), resulting in sufficient nutrients to support growth and meat yield.

Small Intestinal Morphology

The morphology of intestinal mucosa is one of indicative biomarkers to determine gut health. Changes in intestinal mucosa such as decreased villi length or increased crypt depth have been considered to cause tissue damage induced by invading pathogens (Nabuurs et al., 1993). Our results demonstrated that the longer villi of the jejunum were significantly increased

in the LPL05, indicating an increased surface area of the epithelial cells to absorb nutrients for optimal growth and production of broilers. This is in accordance with Khonyoung et al. (2015), who observed the activated cell mitosis in the apical surface of villi in broilers fed lysolecithin. Additionally, the LPL05 had a lower rate of cellular turnover via a shortage of crypt depth both in the jejunum and duodenum than those in NC (Table 4). The defensive mechanism of lysophosphatidylcholine resulted from the considerable secretions of lysosomal enzymes from the mucosal cells and increased shedding enterocytes from villous tips, which allowed permeation of pathogens, toxins, or carcinogens (Tagesson et al., 1985). The diminished crypt depth has been pronounced in lowering the rate of epithelial cell destruction, inflammation, and sloughing of the intestinal segment from bacterial infections (Yason et al., 1987). Our studies found increased villus height and decreased crypt depth in the LPL05, and a high VH:CD ratio also was observed. The increase in jejunal villous height of the middle part is identified as being active in AA absorption in accordance with increased absorptive area of height membrane-bound peptidase activity. In addition, the mature apical enterocyte of the LPL-broiler controls the enterocyte migration, normal sloughing, and greater absorption of nutrients for their growth. These findings were considered to improve broiler performance, nutrient utilization, and anti-inflammation. Even though the crypt depth of birds fed LPL15 was higher than the PC and LPL05 groups, the VH:CD ratio showed no considerable difference, which may attribute to the pathological states of birds as well as intestinal stressors (Choct, 2009). Consequently, the enhanced development of intestinal mucosa may have primary influences on changes of jejunal morphology, absorptive capacity, and defensive mechanism and subsequent effect on bird performance and immunity.

Inflammatory Response and Metabolic Function

Numerous studies have indicated that biosurfactant phospholipids play an important role in the immunological process (Hartmann et al., 2009). Our study also agreed with previous works on a potential lowering effect in the secretion of acute phase proteins, IL-1 and TNF-α, implying that the production of proinflammatory cytokines is inhibited by increasing hemolytic phagocytosis (Zhao et al., 2011). The lysophosphatidylcholine is responsible for increasing vascular permeability in the endothelium during invasion of microorganisms (Qiao et al., 2006), which cause induction of the innate immune system against foreign substances. The key roles of phospholipids in regulating inflammation and innate immunity of cytokines and chemokines were observed by Yun et al. (2005). In vitro study also observed that the LPL can directly integrate in the mucus layer as well as in the membrane of an enterocyte by blocking proinflammatory signals in Caco-2 cells (Nakano et al., 2009). This study showed that it may be possible for LPL to change the barrier properties of the mucosa, therefore alleviating inflammatory response to pathogenic invasion, which is in accordance with morphology criteria.

The LPLs are well established to alter the phospholipid bilayer of cell membranes, which allows the uptake of nutrients across the enterocyte (Lundbæk et al., 2010). This is confirmed by the improvements in metabolic profiles of carbohydrates, proteins, and lipids, similar to previous research (Huang et al., 2008; Han et al., 2010; Zhao et al., 2015). LPL stimulates glucose uptake, which is commonly used as an energy source and a metabolic intermediate in the animal body. The improvement in the availability of glucose also can be found in birds fed a synthetic emulsifier, glycerol polyethylene glycol ricinoleate (Roy et al., 2010). The FFA concentration was greater for birds fed NC than for those fed PC. It might be influenced by modulating the effects of diacylglycerol and triacylglycerol synthesis, which can be hydrolyzed to release FFA (Jansen et al., 2015), so that fat mobilization may increase in response to the demand for energy. However, the LPL15 was shown to reduce FFA level, which was consistent with a previous report (Jansen et al., 2015). According to Huang et al. (2008), the reduction in blood lipid metabolites had significant influence on insulin secretion. This was observed when 2% of crude soybean lecithin was fed to the birds. The insulin has potential in a triglyceride-lowering effect by modulating lipoprotein lipase, which is an important enzyme for the triglyceride hydrolysis (Poonuru et al., 2011). This activation consequently suppresses the secretion of FFA (Jansen et al. 2015). Serum concentration of uric acid decreased linearly in response to increasing the LPL level (Table 5). Uric acid is known as a major end product of N metabolism in broiler chickens, and thus decreased uric acid concentration is considerable to determine AA utilization (Donsbough et al., 2010). The function of modified lecithin in lowering N excretion might be associated with improved digestibility of essential AA as suggested by Han et al. (2010). This is consistent with the longer jejunal villous height and the greater CP digestibility in LPL-supplemented treatments. It is indicated that low nitrogenous diets with LPL addition are sufficient for maximixing amino acid utilization.

Relative Weights of Immune Organs and Carcass Composition

Dietary supplementation of LPL had no significant effect on the relative weight of lymphoid organs, whereas it improved meat yields. This finding was inconsistent with Cho et al. (2012), who found that inclusion of 0.05% sodium steroyl-2-lactylate could increase spleen weight but had less impact on carcass yield when added to diets of 150 kcal/kg less than the commercial

recommendation. The emulsifier source and dietary composition may affect carcass inconsistency results. The current study also found an increase in pancreas size of LPL groups, which was in agreement with Raju et al. (2011), who observed greater pancreas weight in broilers fed 0.5 g/kg lysolecithin. The increase of relative pancreas weight may promote the hydrolysis of triglycerol for greater digestion and absorption of lipids. The significant lowering effect of abdominal fat was not observed in this study. It is possible that LPL alters the facilitation of lipid and protein fractions in the circulation for conversion into the muscles rather than abdominal fat deposition, which consequently affects fatty acid and amino acid deposits in the meat.

Overall, supplementation with LPL on low-nutrient diets improved broiler performance, nutrient digestibility, intestinal morphology, leg muscle, and metabolic profiles, and lowered inflammation. Additionally, the growth performance of LPL birds appeared to be similar to PC.

ACKNOWLEDGEMENTS

We thank Easy Bio Inc. (Seoul, South Korea) for providing the lysophospholipid.

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