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Original Article

Effects of Lysophospholipid and Lipase Enzyme Supplementation to Low Metabolizable Energy Diets on Growth Performance, Intestinal Morphology and Microbial Population and Some Blood Metabolites in Broiler Chickens

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Broiler Chicken, Lipase, Metabolizable Energy, Growth Performance, Intestinal Morphology.

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ABSTRACT

This experiment aimed to evaluate the impacts of dietary lysophospholipid (LPL) and lipase enzyme complementation based on low-energy diets on growth performance, intestinal morphology, blood metabolites, immune response, and carcass traits in broiler chickens. Two hundreds broiler chicks were assigned to a completely randomized design with five treatments and four replications with ten one-day old chicks. The five treatments were: positive control (PC) without LPL supplementation and adequate in all nutrients, negative control (NC) without LPL the reduced 150 kcal/kg of metabolizable energy, NC+ 0.15% LPL (LPL_{15}), NC+ lipase (NCL), NC+ 0.15% LPL+ lipase (NCLL). Feeding LPL improved body weight gain and feed conversion ratio (FCR). In contrast, lipase supplementation showed no significant improvement on weight gain and FCR. Supplementation of LPL and lipase did not have significant effect on immune organ, abdominal fat, and liver and thigh but decreased heart and gizzard and increased breast relative weight ($p< 0.05$). Digestibility of dry matter did not show significant effect but crude protein and ether extract improved digestibility in LPL_{15} and NCLL group in contrast to NC group ($p<0.05$). Dietary treatment showed no significant improvement on the metabolic blood factors ($p<0.05$). The inclusion of LPL to negative diet (LPL_{15}) and LPL+lipase to negative control diet raised villus height, ratio of villi height to crypt depth and increased crypt depth. Overall, LPL inclusion to diet increased weight gain and improved FCR, crude protein and fat digestibility, and improved villus height and ratio of villi height to crypt depth to NC group.

INTRODUCTION

The high growth rate of broiler chicks increases their need for energy and protein sources to support growth and performance. Because of the high concentration of energy in the diet, oils and fats are generally added to diets to fulfill this requirement. The digestibility of fats and oils is dependent upon their chemical and physical structures. One of the factors influencing the energy metabolism of fats for poultry is the degree of saturation of fatty acids and their chain length (Smink *et al*., 2010). Generally, the digestibility of oil and fat decreased with increasing the chain length and degree of saturation of fatty acids. Increasing the digestibility of these substances reduces fat or oil level in the diet by sustaining a similar level of performance (Ho Cho *et al*., 2012). Lysophospholipid is a natural surfactant derived from the soy lecithin, enzyme hydrolysis by removing a fatty acid from the phospholipids through the phospholipase A2 (Joshi *et al*., 2006). Increasing the digestibility of fats or oil reduces their dietary intake and, ultimately, decreases dietary cost. LPL changes the number and pores of the cell membrane, increases the flux rate of macromolecules across the cell membrane and, consequently, increases the efficiency of the

energy (Lundbak *et al*., 2010). In this way, LPL was able to carry the substances from small particles like calcium or large components like polysaccharides to be decomposed for absorption. Boontiam *et al*. (2017) described that the performance, nutrient digestibility, and intestinal morphology were enhanced following the LPL supplementation on low-energy diets.

In addition to increase the role of LPL, roles such as immune stimulation can be mentioned (Lewis *et al*., 2016). Lewis *et al*. (2016) demonstrated that phosphatidylcholine LPL has a contribution to regulating cell multiplication and secretion of interleukin, and consequently promoting cell mediated immunity. Based on these findings, our hypothesis was that LPL could be a proper substance to compare with lipase enzyme on the broiler performance and nutrient exploitation. Accordingly, the aim of this experiment was to investigate the impacts of lysophospholipid and lipase enzyme supplementation to lower metabolizable energy diets on growth performance, intestinal morphology and microbial population, and blood metabolites in broiler chickens.

MATERIAL AND METHODS

The entire processes applied in this study underwent the approval of the Animal Care Committee at Sari Agriculture and Natural Resources University (SANRU) of Iran**.**

Experimental Design, Diets, and Management

200 one-day old male chicks were obtained from a local hatchery (Homa farm, Iran). All chicks were housed in pens (130 × 100 cm; length × width) and supplied with food and water *ad libitum*. Room temperature was set within the first three days and was gradually decreased to 24 °C until the end of the experiment; the lighting program consisted of 23 h of daily light throughout the experiment in accordance to the management guidelines of commercial broilers. All pens were equipped with tube feeders and automatic drinkers. Chicks were generally reared on floors that covered with wood shavings. Two hundred broiler chicks (Ross 308) were allocated to a completely randomized design with five treatments and four replicates with ten one-day old chicks. The five treatments were: positive control (PC) without LPL supplementation and adequate in all nutrients, negative control (NC) without LPL the reduced 150 kcal/kg of metabolizable energy, NC+ 0.15% LPL (LPL_{15}), NC+ lipase (NCL), NC+ 0.15% LPL+ lipase (NCLL). The LPL was obtained from Sinason Inc., (Lipidopepin, Mazandaran, Iran). The formulation of the experimental diet was based on nutrient needs for Ross 308 broilers (Aviagen, 2009; Table 1) to 3 period (0-10 starter, 11-24 grower, 25-marker finisher).

The weights of all the birds were measured and records of feed intake were taken on days zero, 10, 24, and 37 to calculate body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). On days 19, 20 and 21 clean excreta were collected twice a day from the birds that fed with chromic oxide (0.3%) as an indigestible marker to estimate the digestibility of organic matter, fat and nitrogen (Cohn *et al*., 2010). Form day 16 to 18, the adaptation diet that mixed with chromic oxide (0.3%) were assigned to treatment. Five excreta samples were collected and pooled per pen and stored frozen at –20°C for further examination. On days 21 and 38, two blood samples were taken from the left bronchial vein into a sterile tube that included EDTA. Subsequent to collecting blood, the samples were relocated to the laboratory and the plasma harvest was frozen for further analysis. At the completion of the experiment, two broilers from each replicate were weighed individually and then sacrificed by cervical dislocation. The organs such as gizzard, abdominal fat, breast, thigh, liver, heart, spleen, and bursa of fabricius were taken from each broiler. At the end of the experiment, the middle part (about 5 cm piece) of each jejunum per replicate was collected from one of the same broilers that was selected for carcass characteristics.

At the termination of the feeding trial (38 d), the ileum track of two of the slaughtered birds (used for carcass characteristics) was immediately removed. The ileum was dissected longitudinally by a sterile scissor, and digesta samples were gathered instantly in pre-weighed 20-mL sterilized plastic tubes and transported to the laboratory to count the number of total bacterial, coliforms and lactobacillus. At 24 d of age, two broilers were chosen per pen and assigned to sheep red blood cell (SRBC). Seven and 14 d after SRBC administration, the broiler that received SRBC were bled via the brachial vein into tubes with EDTA as the anticoagulant to test antibody titer.

Laboratory Analysis

To collect the samples such as, scales, feather, and filoplumes first the fecal material were removed; then, they were kept frozen at –20 °C. All of the pooled samples were allowed to dry in an oven for 72 h under 60 °C. The digestibility of the feed analyses was carried

Table 1 – Chemical composition and nutrient content of the experimental diet.

Ingredients (%)	Starter 0-10 d		Grower 11-24 d		Finisher 25-42 d	
	PC	NC	PC	NC	PC	NC
Corn grain	46.81	51.66	50.47	58.00	56.25	62.61
Soybean meal	44.57	42.76	40.50	36.45	34.95	31.53
Soybean oil	4.37	1.37	5.20	1.79	5.18	2.45
DCP	1.67	1.16	1.47	1.39	1.34	1.26
Limestone	1.17	1.69	1.07	1.04	1.00	0.97
Mineral premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.26	0.23
NaHco3	0.15	0.10	0.20	0.20	0.20	0.20
DL-Met	0.35	0.35	0.29	0.27	0.26	0.23
L-Lys	0.12	0.12	0.04	0.09	0.06	0.02
L-Theronine	0.04	0.04	0.01	0.02	0.00	0.00
Calculate feed Analysis						
ME(kcal)	2950	2800	3050	2900	3150	3000
CP (%)	22.62	22.62	21.15	21.15	19.20	19.20
Ca (%)	0.94	0.94	0.85	0.85	0.78	0.78
Av. P (%)	0.47	0.47	0.42	0.42	0.39	0.39
Lys (%)	1.42	1.42	1.26	1.26	1.14	1.14
Met + cys(%)	1.06	1.06	0.97	0.97	0.89	0.89
Theronine	0.95	0.95	0.86	0.86	0.77	0.77

1: Composition (per kg): manganese, 75,000 mg; iron, 50,000 mg; copper, 8,000 mg; iodine, 750 mg; . 60,000 µg; selenium. 2: Composition (per kg): 0.3 mg/kg vitamin A, 8,000 IU; vitamin D3, 2,000 IU; vitamin K3, 1,800 mg; vitamin B1; 1,800 mg; vitamin B2, 6,000 mg; vitamin B6, 2,800 mg; vitamin B12, 12,000 µg; pantothenic acid, 10,000 mg; niacin, 40,000 mg; folic acid, 1,000 mg; biotin.

out according to the protocol of AOAC (2000) to determine the digestibility of crud protein digestibility of CP by the Kjeldahl (method 984.13), ether extract (EE) by the Soxhlet (method 920.39), and DM (method 930.15). Next, the dry matter, crud protein, and fat in the feed and feces were put for analysis and their values were used to determine the apparent total tract digestibility. The samples from the jejunum were fixed in 10%10% (vol/vol) neutral buffered formalin solution and embedded in paraffin wax. Tissues from the segment kept in 10% neutral form aldehyde solution were cut into five cross sections (roughly 5μm thickness) that were further stained by haematoxylin and eosin. Villous height was determined from the villous apical to crypt junction, but crypt depth was determined from the villous bottom to the crypt, followed by calculating the ratio of villous height to crypt depth (VH: CH ratio). A light inverter d microscope (TE Nikon 300, Japan) was used for morphological studies.

Blood plasma was harvested with a centrifuge at 3,000-× g for 15 min. Then, the plasma was preserved for further analysis at –20 °C. The total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG), very low density lipoprotein (VLDL), glucose and uric acid in the plasma samples were analyzed with an autoanalyzer (Automatic Biochemical Analyzer, Mindray. BS-120). The content sample of the ileum was accomplished for subsequent analysis of the bacterial populations by serial dilution. For this propose, serial dilutions (10–2 to 10–7) of the intestinal digesta were prepared with PBS prior to inoculating onto petri dishes of sterile agar. Lactobacilli were grown on Man Rogosa and Sharp (MRS) (Merck, Germany), and the samples were plated on Mac Conkey agar media (Himedia, India) to enumerate Coliforms. Total aerobic bacteria were enumerated using nutrient agar media.

Statistical analysis

The experimental data were analyzed by the variance procedure of statistical analysis system (SAS 2003) based on a completely randomized design. Mean values were assessed by Duncan's new multiple range test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Growth performance

Data of hatchability, BW, BWG, FI, and FCR are represented in Table 2. Significant effects were observed on BWG and FCR during the starter, growing,

Table 2 – Effects of LPL supplementation to lower nutrient diets on growth performance in broiler.

Criteria	PC	NC	LPL15	NCL	NCLL	SEM	*p*-value
BWG (g/bird)							
0 to 10 d	207.7^{a}	168.2^{bc}	163.4^{c}	165.8^{c}	174.6^{b}	4.0069	0.001
11 to 24 d	877.2^{a}	797.2^{c}	853.1^{b}	840.4^{bc}	891.0^{a}	16.005	0.007
25 to 38 d	1455.2^{a}	1339.2^{b}	1478.4^{a}	1341.7^{b}	1430.9^{ab}	30.388	0.001
0 to 38 d	2540.2^{a}	2304.6^{c}	2494.9^{ab}	2347.9^{bc}	2496.5^{ab}	16.799	0.024
FI (g/bird)							
0 to 10 d	221.00	207.90	202.18	214.06	218.50	6.881	0.125
11 to 24 d	1249.63	1210.63	1168.13	1170.31	1220.31	18.187	0.290
25 to 38 d	2226.5	2198.6	2225.3	2150	2189.6	41.361	0.189
0 to 38 d	3697.13	3617.13	3595.61	3534.38	3628.41	22.143	0.078
FCR							
0 to 10 d	1.06^{c}	1.23^{b}	1.23^{b}	1.35^{a}	1.25^{b}	0.0283	0.001
11 to 24 d	1.41^{b}	1.51^{a}	1.36^{c}	1.39^{c}	1.31^{d}	0.0154	0.001
25 to 38 d	1.53^{b}	1.64^{a}	1.51^{b}	1.60^{a}	1.53^{b}	0.0144	0.003
0 to 38 d	1.45^{b}	1.56^{a}	1.44^{b}	1.50^{ab}	1.45^{b}	0.0193	0.0017

Positive control (PC), negative control (NC), NC+ 0.15% LPL (LPL_{15}), NC+ Lipase (NCL), NC+ 0.15% LPL+ Lipase (NCLL).

BWG: Body Weight Gain

FI: Feed Intake

FCR: Feed Conversion Ratio Villus Height 2: Crypt Depth

^{a,c} Means in a same column with different superscripts significantly differ (p<0.05).

and finishing phases and throughout the whole experiment (p<0.05). The broilers that received dietary LPL supplement gained less BWG and greater FCR in the starter and growing phase than PC (p<0.05). From d 11 to 24, BWG rose (p<0.05) by LPS complementation and the best FCR was achieved in this group (p<0.05). Supplementation of LPL led to improvements in BWG and FCR during growing phase (p<0.05). During the overall period, increases (p<0.05) for BWG and decreases for FCR were noticed in broilers fed LPL and LL in comparison to NC diet, same as PC diet (p<0.05).

Diet energy is channeled towards maintenance and production. Decreased diet energy had effects on the maintenance and growth performance in broiler. Nevertheless, findings of the present study showed that FI between broilers fed basal diet was not different. A study performed by Richards (2003) showed that broilers chosen for fast weight increment and muscular mass accretion and the hens were not able to adjust the voluntary FI appropriately in accordance with energy levels, which may explain the unchanged FI herein. In contrast, reduced FI through energy-deficient diet (150 kcal/kg ME lower) was observed only from day 22 to 35 in broilers (Cho *et al*., 2012).

It is believed that LPL supplementation is beneficial for broilers and will activate several key functions in the body under restricted energy and nitrogenous diets for adequate nutrient supply. Sabiha (2009) stated that the LPL increases the nutrient digestion and absorption by their capability for formation of relatively smaller micelles and by enhancing the flux rate of different digested nutrients throughout the cell membrane by augmenting its penetrability. Based on this feature, the LPL is able to possess the permeability of cell membrane and work together with bile salts during the first stages of fat digestion. Wang *et al*. (2016) demonstrated that sodium stearoyl-2-lactylate incorporation in a diet with less-energy led to partial improvement in the growth performance. Studies by Cho *et al*. (2012) and Zhang *et al*. (2011) show that BWG of broilers was increased by lysolecithin; it also tended to decrease FCR from day one to 21, which is consistent with the observations of the present study in that LPL increased BWG and decreased FCR comparing to negative control diet.

Nutrient digestibility

Nutrient digestibility of the dry matter, crud protein, ether extract, and the intestinal morphology are summarized in Table 3. The experimental diets could not significantly affect the dry matter. The digestibility of crud protein and ether extract was affected when LPL, LPS, and LL supplemented to diet (p<0.05) compared to NC group, whereas this effect was more significant for LPL than LPS.

In this research, LPL_{15} supplementation increased the digestibility of ether extract and crud protein in broilers. The effect of lysophospholipid on nutrient digestibility depends on different sources in the basal diet, composition and incorporation rate of dietary fats might result in discrepant outcomes (Zhang *et al*., 2010;

Table 3 – Effects of LPL supplementation on nutrient digestibility and intestinal morphology in broiler chickens.

Criteria	PC	NC	LPL15	NCL	NCLL	SEM	*p*-value
Digestibility (%)							
Dry matter	68.081	70.781	69.396	68.832	68.081	2.213	0.22
Crude protein	58.566^{a}	48.976^{b}	60.831^{a}	53.639^{ab}	61.182^{a}	2.565	0.002
Ether extract	80.754^{ab}	72.170^{b}	84.994^{a}	80.014^{ab}	87.992^{a}	3.788	0.025
Intestinal Morphology(µm)							
VH[1]	759^{b}	763^{b}	940^{a}	686^{b}	943^{a}	27.4	0.10
CD[2]	88.7^{c}	105^{bc}	128^{a}	112^{ab}	119^{ab}	6.97	0.460
VH:CD ratio	8.93^{a}	7.24^{ab}	7.38^{ab}	6.12^{b}	8.09^{ab}	0.720	0.280

positive control (PC), negative control (NC), NC+ 0.15% LPL (LPL_{15}), NC+ Lipase (NCL), NC+ 0.15% LPL+ Lipase (NCLL).

[1]: Villus Height [2]: Crypt Depth

[a,c]Means in a same column with different superscripts significantly differ (p<0.05)

Zhao *et al*., 2015). Han *et al*. (2010) suggested that the inclusion of lysolecithin at 0.10% in the basal diet increased the digestibility of nitrogen and the energy values in laying hens. Also Zhang *et al*. (2010) did not observe significant effects of lysophosphatidylcholine on digestibility of dry matter and crude protein in broiler chickens. LPL supplementation enhances the nutrient digestibility by combining digesta, decreasing the size of the emulsion droplet, and making lipids accessible to the enzyme to the gastrointestinal tract. Hence, it could be said that lipid absorption was boosted by LPL through their presence in micelle creation (Sabiha, 2009).

Lysophospholipids alter phospholipid (bi) layers of enterocytes cells. Lysophospholipids are known to increase the fluidity and permeability of the membrane that can also persuade modifications in protein channel formation and raising ion exchanges, with direct impacts on the stability of the cell membrane by decreasing deformation energy (Lundbaek *et al*., 1994). Both of these changes promote the intake of nutrients throughout the enterocyte membrane giving rise to a greater dietary nutrient bioavailability (Sugawara *et al*., 2001). The effects of lipase supplementation on lipid digestibility are dependent on source and type of lipids, the composition of the diets, and the age of birds. Meng *et al*. (2004) showed that fat digestibility did not improve with lipase supplementation (100 U/kg feed) into tallow-supplemented diets in broilers.

Intestinal morphology

No significant effects were observed with LPL, LPS, and LL incorporation in all conditions of intestinal morphology (Table 4) but significant differences were observed in Duncan analysis for intestinal morphology. Villous height and crypt depth increased when LPL and LL supplemented to diets but the lowest VH: CH ratio was achieved in LPS treatment (p<0.05).

Increasing the surface area for digestion and absorption is an important factor to express their performance in birds. It is believed that the morphology of intestinal mucosa is one of the indicators for determining gut health. The improvement in villus height and villus height to crypt depth ratio increased absorptive area and improved digestive enzyme function. The lower VH: CD ratio in broiler fed with the NC diet resulted in an increase tissue turnover of the duodenal mucosa. A higher turnover rate causes

Table 4 – Effects of LPL supplementation on carcass weights in broiler chickens at 38 d of age.

Criteria	PC	NC	LPL15	NCL	NCLL	SEM	*p*-value
Carcass traits (% to BW)							
Empty weight	67.33	65.08	66.09	63.72	65.06	0.702	0.81
Breast	26.82^{a}	23.08^{b}	25.72^{a}	23.21^{b}	26.28^{a}	0.007	0.03
Thigh	18.24	19.62	19.33	19.50	18.78	0.004	0.23
Gizzard	2.22	2.23	2.08	2.24	2.18	0.002	0.43
Heart	0.05	0.48	0.47	0.49	0.47	0.003	0.58
liver	1.20^{b}	1.40^{a}	1.08^{b}	1.21^{b}	1.22^{b}	0.005	0.05
Abdominal fat	0.84^{b}	1.52^{a}	0.72^{b}	0.81^{b}	0.90^{b}	0.002	0.01
Immune organs (% to BW)							
Spleen	0.07	0.08	0.08	0.09	0.07	0.008	0.69
Bursa	0.11	0.14	0.19	0.16	0.17	0.002	0.06

Positive control (PC), negative control (NC), NC+ 0.15% LPL (LPL_{15}), NC+ Lipase (NCL), NC+ 0.15% LPL+ Lipase (NCLL).

[a,c]Means in a same column with different superscripts significantly differ (p<0.05)

higher maintenance requirement, which can finally lead to retarding broiler performance (Khongyoung *et al*., 2015). Nevertheless, studies show that the LPL reduced the rate of cellular turnover by reducing the crypt depth in the jejunum. Moreover, the mature apical enterocyte of the broilers receiving LPL was able to control the enterocyte migration, and normal sloughing (Khongyoung *et al*., 2015). Some studies argued that the enzyme supplementation influences the gut morphology in birds positively (Liu & Kim, 2016).

The outcomes of this investigation show that the villus height of the jejunum considerably increased in the LPL15 group suggesting a rise in the surface area of the epithelial cells; it may also show enhanced nutrients absorption for optimum growth and production of broilers. The above observations correspond to that of Khonyoung *et al*. (2015) arguing that the cell mitosis has been activated in the apical surface of villi in broilers fed lysolecithin. Moreover, this may also justify the enhanced nutrient digestibility as it boosted the digestive and absorptive capacity of the small intestine. It has been reported that there is an association between villus heights and the absorption capacity of the enterocytes, and it is probable that the short villi decreases the surface area for nutrient absorption (Parsaie *et al*., 2007). Studies have shown that addition of lipase (0 to 11,250,000 U/kg feed) to broilers diets containing 4% blended fat (animal-vegetable) did not no influence the gut morphology (Al-Marzooqi & Lesson, 2000).

Relative Weights of Carcass Components and Immune Organs

The results of Table 4 shows how various treatments affect the relative weights of carcass weights and immune organs in broiler chickens. As it is shown in this Table, broiler chickens fed with a restricted energy (150 kcal/kg diet lower than NC diet) and the inclusion of LPL and LL had greater breast weight than NC and LPS such as NC diet ($p< 0.05$). The addition of LPL to the restricted energy diet resulted in higher liver weight than other treatments ($p< 0.05$). In addition, the relative abdominal fat was markedly higher in NC diet that in other treatments ($p< 0.05$). The relative weights of spleen and bursa of Fabricius did not change significantly among dietary treatments (Table 4).

In line with these results, Boontiam *et al*. (2017) presented evidence that LPL-supplemented diet could not significantly influence the relative weight of lymphoid organs. Wang *et al*. (2015) reported that abdominal fat increased with the addition of 0.05% sodium stearoyl-2-lactylate in low energy diet. Adding soy lecithin supplement (0.25%) improved carcass quality and raised abdominal fat content on day 40 (Boulos *et al*., 2011). Various results have been reported on abdominal fat content that might be caused by the emulsifier source, improved level, and dietary composition. As inferred from our findings, the complementation of lipase in decreased energy diet additionally reduced the abdominal fat in comparison to the negative control diet. Al-Marzooqi & Lesson (2000) argued that the percentage weight of the liver enhanced following lipase complementation (0 to 11,250,000 U/kg feed) in broilers fed diets enriched with 4% animal-vegetable mixed fat at 21 d, and it is likely that this increase in the liver weight is associated with boosted metabolic activity of lipid utilization.

Sabiha (2009) reported that LPL lowered liver fat percentage suggesting enhanced digestion and absorption of fat that can result in lean meat production in broilers.

Plasma profile and immune response

Plasma profile such as glucose, cholesterol, triglycerides, HDL, LDL, VLDL, uric acid and, immune response such as total titer against SRBC (sheep red blood cell), IgG, and IgM are summarized in Figures 1 and 2. There is no significant effect of treatment on the plasma profile and immune response in broiler chickens. There was an increment tendency for IgM unlike IgG and total titter (p=0.082).

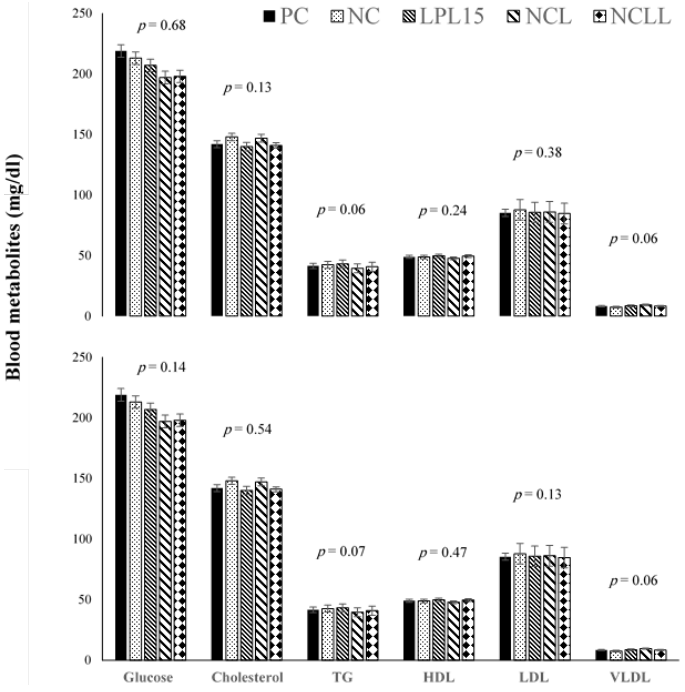

Figure 1 – Effects of LPL supplementation on blood metabolites in broiler chickens at 21 (Top) and 38 (Bottom) days of age. Positive control (PC), negative control (NC), NC+ 0.15% LPL (LPL_{15}), NC+ Lipase (NCL), NC+ 0.15% LPL+ Lipase (NCLL).

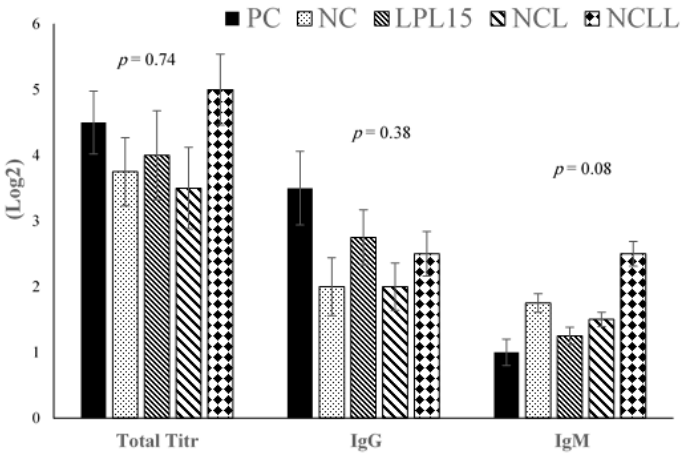

Figure 2 – Effects of LPL and LPS supplementation on specific antibody titers to SRBC (log2) at 35 d of age. Positive control (PC), negative control (NC), NC+ 0.15% LPL (LPL15), NC+ Lipase (NCL), NC+ 0.15% LPL+ Lipase (NCLL).

In agreement with our result, no differences were observed in serum triglycerides, total cholesterol, HDL, and LDL of broiler on day 35 in low energy diet that was enhanced by sodium stearoyl-2-lactylate supplementation (Wang *et al*., 2016). Total cholesterol concentrations were decreased by LPL supplementation diet compared with basal diet treatments on day 42 (Malapure *et al*., 2011). Cohn *et al*. (2010) observed more than 50% suppression of cholesterol uptake with the addition of 15 mg lecithin**.** Roy *et al*. (2010) noticed that dietary complementation of an emulsifier declined LDL and total cholesterol concentration on day 20; however, no differences were observed on day 39. According Zhao *et al*. (2015), LPL incorporation reduced the concentrations of LDL, total cholesterol, and triglycerides on day 14, that argument can be that, an association between the faster rates of absorption and metabolism of ingested fat and lower serum triglycerides in pigs fed lecithin; it is likely that the chylomicrons were either removed from the blood at a faster rate or secreted into the blood at a slower rate.

Hu *et al*. (2017) concluded that adding lipase supplementation into decreased energy diets resulted in enhanced activities of pancreatic lipase and reduced TG and LDL levels in broilers. It is believed that for lower serum TG in broilers fed lipase is probably caused by faster rates of absorption and metabolism of ingested fat may be the reason for lower serum TG in broilers fed lipase.

Population of intestinal total aerobic bacteria, coliforms, and lactobacilli

The microflora compositions of the Ileum on day 42 are detailed in Table 5. No differences were found in the viable counts of total Lactobacillus in ileum. The lowest population of total aerobic and coliform bacterial was observed in NC diet ($p<0.05$). The chickens supplemented with LL and LPL had higher total aerobic and Coliform bacterial population same as PC diet ($p<0.05$).

The amount of microorganisms affects the bile salt integrity by the bacterial cholyltaurine hydrolase activity (Knarreborg *et al*., 2010), which affects the digestibility and performance of chickens. Huyghebaert *et al*. (2011) reported that lysophospholipids could contribute to the reduction of the amount of growth-depressing metabolites generated by gram-positive bacteria, which is one of the beneficial factors attributed to the antibiotic utilization. This decrease can be explained by the antimicrobial effects of lysophospholipids, which may have occurred in two ways. The lysophospholipids may have acted directly on the gram-positive microorganisms altering the cell membrane permeability, leading to damage in the bacteria integrity through ionic imbalance (Silva, 2009). The second cause is related to lower amounts of substrate in the intestinal lumen.

Table 5 – Effects of LPL and LPS supplementation on intestinal population (log10 cfu/g).

Criteria	PC	NC	LPL15	NCL	NCLL	SEM	*p*-value
Total aerobic bacterial	8.62^{ab}	7.86^{c}	8.57^{ab}	8.20^{bc}	8.72^{a}	0.149	0.005
Coliforms	7.84^{a}	7.09^{c}	7.62^{ab}	7.36^{bc}	7.87^{a}	0.102	0.003
Lactobacillus	7.02	7.20	7.02	7.04	7.02	0.159	0.910

Positive control (PC), negative control (NC), NC+ 0.15% LPL (LPL_{15}), NC+ Lipase (NCL), NC+ 0.15% LPL+ Lipase (NCLL).

^{a,c}Means in a same column with different superscripts significantly differ ($p<0.05$).

CONCLUSION

The addition of a lysophospholipid-based emulsifier to the diet significantly improved FCR and BW gain during growing phase of broiler chickens. Lysophospholipid supplementation increased digestibility of crud protein and ether extract in broiler. Villous height and crypt depth increased when LPL and LL were supplemented in the diets. In our results, the amounts of glucose**,** serum triglycerides, total cholesterol, HDL, and LDL, VLDL, and Uric acid were not different in experimental broilers.

COMPETING INTERESTS

The authors declared that they have no competing interests.

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