

Effect of dietary lysophospholipid supplementation on growth performance, serum lipids, small intestine morphology and caeca microflora in broiler chickens

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Abstract

Background: The digestibility of animal fats and oils is limited by a reduction in the production and secretion of lipase and bile salts in young chickens. The addition of a natural emulsifier (lysophospholipids [LPL]) in poultry diet may increase the emulsification of lipids and their digestibility.

Objectives: An experiment was conducted to evaluate the effects of feed LPLs supplementation with different fat sources on performance, serum lipid composition, small intestine morphology and caeca microflora in broiler chickens.

Methods: A completely randomized factorial design ($2 \times 3 \times 2$) was used to evaluate the effect of LPL supplementation (0 and 0.25 g/kg) and three different fat sources (soybean oil, tallow and a 50:50 mixture of the two) in corn and soybean meal diets containing two levels of fat (1.5 and 3%), providing 12 isocaloric and isonitrogenous grower diets. Each experimental diet was fed to six replications of 10 birds from 15 to 28 days of age. Average growth performance during this period and small intestine morphology, serum lipid composition and caeca microflora were evaluated on day 28.

Results: The interaction effects of LPL supplementation, source and/or level of fats were not significant for the performance parameters measured during the 15 to 28 days. The treatment effects were significant for the villus width and crypt depth measured in the jejunum on day 28. The LPL supplementation significantly increased crypt depth. The interaction effect of fat source and level of fat were significant for villus width. The addition of a 3% blend of soybean oil/tallow (50/50) reduced the serum low-density lipoprotein (LDL) level. The *Lactobacillus* population was increased by the addition of LPL, or a 1.5% blend of soybean oil and tallow, to the diet.

Conclusions: Our study showed that LPL supplementation of diet containing a 1.5% blend of soybean oil and tallow can improve serum lipid indices and caeca *Lactobacillus* populations in broiler chickens.

KEYWORDS

broiler chickens, performance, lysophospholipid, soybean oil and tallow

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1 | INTRODUCTION

The addition of different fats and oils has an important role in poultry nutrition and performance. Many investigators were looking to find new methods to increase feed productivity in animals. Corn is a major source of energy supply in poultry diets. The increasing demand for corn would lead to an increase in its future prices (FAO, 2009). Therefore, it is possible to provide part of the diet energy through other available energy sources. Fat is a higher-energy dense ingredient for birds, and it is generally used to provide high-energy diet. The metabolisable energy of a diet can be increased through the addition of a fat to diet, because fat produces more than twice energy per gram compared to carbohydrates or protein sources. Digestion and absorption of dietary fat requires hydrolysis of water-insoluble triglycerides. Dietary fats are emulsified by bile salts and are hydrolysed by pancreatic lipase (Zhao et al., 2015). Thus, this results in the generation of free fatty acids and monoglycerides on the surface of oil and water. Bile salts also play a key function in the configuration of mixed micelles, which are then absorbed by the mucosal cells of the small intestine (Kragdahl, 1985). Digestion of lipids depends on various factors such as sources and types of lipids, diet composition and age of the bird.

The digestion and absorption processes are very important because of the small size of the digestive system in poultry, feedstuffs remain in the digestive tract of poultry for about 2 h until digestion and absorption are completed. The capacity to digest and absorb lipids is poorly developed in young birds and depends on physiological maturity, a low level of lipase production (Al-Marzooqi & Leeson, 1999), intestinal microflora status and diet composition (Maisonier et al., 2003), the ratio of unsaturated fatty acid (UFA) to saturated fatty acids (SFA) in the diet (Ketels & Groote, 1989), the presence of pentosans (Choct & Anison, 1992) and dietary fibre (Jimenez-Moreno et al., 2009).

SFAs in the diet probably reduce the formation of stable mixed micelles in the small intestine lumen, which reduces the fat digestion and absorption in young birds (Leeson & Atteh, 1995). After hatching, (A) the gastrointestinal tract of the chick is still immature, (B) also, digestive and absorption processes are incomplete in young birds, (C) on the other hand, in adult birds, the mean retention time of dry matter is limited in the small intestine, (D) finally, there is probably no accurate supply of required nutrients at the amount of feed intake (FI). These factors can cause to reduce the growth performance and production index of the birds. Apparent digestibility of fats is low in young chicks due to saturated fats, secretion and incomplete recycling of bile salts and insufficient production of fat-carrying gut proteins during the early growth of birds. Therefore, feed additives that improve the digestion and absorption of fat can be used in diets to provide more energy per unit of feed volume for broilers, and emulsifiers are of this type (Xing et al., 2004).

Emulsifier acts as a catalyst to increase the active surface of fats, thereby enhancing the action of lipase, which can help to hydrolyse triglycerides into monoglycerides and fatty acids, so it favours the formation of micelles including of lipolysis products (Upadhaya et al., 2017). Lysophospholipids (LPLs) are produced by the activity of the

enzyme phospholipase A2. Because LPLs have a critical micelle concentration (CMC) of 0.02–0.2 mM/L, which is 20–200 times more effective than bile (CMC = 4 mM/L) and lecithin (CMC = 0.3–2 mM/L), they can spontaneously form very small micelles (Langmuir, 2002). This showed that LPLs are a better source as an exogenous emulsifier as they have a higher emulsifying and micelle-forming capacity than bile salts and lecithin (Zhang et al., 2011).

Therefore, the objective of the present study was to estimate the effects of three fat sources (soybean oil, tallow and a 50:50 mixture) with or without LPLs supplementation on FI, body weight gain (BWG), feed conversion ratio (FCR), serum lipid composition, small intestine morphology and caeca microflora in broiler chickens.

2 | MATERIALS AND METHODS

The experimental protocols describing the management and care of animals were reviewed and approved by the guidelines of the Iranian Council of Animal Care (1995).

2.1 | Experimental design, diets and management

A total of 1000-day-old Ross 308 broiler chicks with an average initial body weight (BW) of 39.15 ± 0.66 g were purchased from a commercial supplier. Standard diets were based on corn and soybean meal and formulated based on the National Research Council [NRC] (1994) or Ross 308 strain catalogue (2019) recommendations and fed to broiler chicks for the starter and grower periods (Table 1) from 1 to 10 and 11 to 14 days of age, respectively. Seven hundred twenty of them were weighed in a group of 10 (with an average BW of 303.44 ± 0.5 g/b) and randomly allocated to 72 floor pens (1.2×1.2 , m \times m) on day 14. The 12 dietary treatments with a $2 \times 3 \times 2$ factorial design, including two levels of LPL (0 and 0.25 g/kg of LPLs, Artifier), three fat sources (soybean oil, tallow and a 50:50 mixture of the two) and two levels of fat (1.5 and 3%), were provided, and each was randomly fed to birds in six replicate pens from 15 to 28 days of age. Temperature and light programs were maintained according to the Ross 308 guide (2019). The experimental diets during the grower period (15 to 28 days) were formulated to meet or exceed the nutrient requirements for Ross 308 broilers (Table 2). The diets were formulated on the isonitrogenous and isocaloric basis. Water and mash feed were provided ad libitum. The LPL was added on 'top of' the diet according to each treatment (Table 2). The apparent metabolisable energy value of the soybean oil, tallow and their 50:50 mixture used to formulate the diets obtained from the study by Salari et al. (2020) were 8367, 6134 and 6547 kcal/kg, respectively.

2.2 | Small intestinal morphology

One bird was randomly selected from each pen on day 28. Chickens were slaughtered by cervical dislocation. After slaughtering, approximately 3 cm of the middle portion of the jejunum (the midway between

TABLE 1 The standard commercial starter and grower diets fed to all birds from 1 to 10 and 11 to 14 days^a of age, respectively (as-fed basis).

Diet ingredients	Starter (0–10 d)	Grower (11–14 d)
Corn	49.36	52.69
Soybean meal (44% CP)	41.53	37.78
Oil	4.49	5.37
Dicalcium phosphate	1.93	1.71
Limestone	1.06	0.98
Common salt	0.28	0.28
D,L-methionine	0.37	0.32
L-lysine hydrochloride	0.24	0.17
L-threonine	0.11	0.07
Vitamin and mineral premix ^b	0.5	0.5
NaHCO ₃	0.12	0.12
Choline chloride	0.01	0.01
Calculated composition%		
Metabolisable energy (kcal/kg)	3000	3100
Crude protein (%)	23	21.5
Calcium (%)	0.96	0.87
Available phosphorous (%)	0.48	0.43
Lysine (%)	1.44	1.29
Methionine (%)	0.71	0.64
Methionine + Cystine (%)	1.08	0.99
Threonine (%)	0.97	0.88
Sodium (%)	0.17	0.17
Chlorine (%)	0.18	0.18

^aLeeson et al. (1996); El-Wahab et al. (2020).

^bAmounts per kilogram diet: vitamin A (trans retinyl acetate), 12500 International units; vitamin D₃ (cholecalciferol), 5000 International units; vitamin E (tocopheryl acetate), 80 mg; vitamin K₃, 3.2 mg; thiamine, 3.2 mg; riboflavin, 8.6 mg; panthothenic acid, 8.6 mg; pyridoxine, 4.86 mg; B₁₂ Cyanocobalamin, 0.02 mg; niacin, 62.51 mg; biotin, 0.25 mg; folic acid, 2.2 mg; antioxidant, 2.5 mg. Mineral premix supplied per kilogram diet: Fe, 20.23 mg; Mn, 120 mg; Zn, 110 mg; Cu, 16 mg; I, 1.252 mg; Se, 0.3 mg. Choline chloride, 300 mg (vitamin and mineral supplements).

the point of entry of the bile ducts and Meckel's diverticulum of the jejunum) was excised and fixed in 10% cold phosphate-buffered saline. Three cross-sections from each sample were prepared after staining with haematoxylin and eosin using the standard paraffin embedding procedures. Histological examinations were carried out according to the method of Iji et al. (2001).

2.3 | Serum lipid composition

Prior to slaughtering one bird from each replicate group, 3 mL of blood from the brachial vein was collected into a tube, and the serum was

collected by centrifugation at 3000 revolutions per minute at 18°C for 10 min (Rotofix 32A centrifuge, Hettich) The serum supernatants were subsequently transferred into 1 to 1.5 mL centrifuge tubes and stored at –20°C until analysis. The serum composition of total triglycerides, cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) was analysed by an automatic biochemical analyzer (Technicon RA-1000), following the instructions of the corresponding reagent kit (Pars Azmon Co.).

2.4 | Caeca microflora

The caeca content was collected from the same birds slaughtered at 28 days of age; each sample was homogenised with 1 mL of serum physiologic and serially diluted, and then plated onto selective agar medium for enumeration of target bacterial groups (Owens et al., 2008). Enumeration of the *Lactobacilli* bacteria was performed on man-rosgosa-sharpe (MRS) agar media and incubated for 48 h at 30°C under anaerobic conditions. *Escherichia coli* were grown on MacConkey agar (Beijing Aoboxing Bio-tech Co. Ltd.). All sample plates were incubated at 37°C for 24 h. Bacteria were counted by optical enumeration of colonies, using the most frequent collection from dilutions that resulted in 35 to 300 colonies per plate. The caeca microflora enumerations were demonstrated as base-10 logarithm colony-forming units per gram of caeca content.

2.5 | Statistical analysis

The study was conducted as a 2 × 3 × 2 factorial in a completely randomised design. Data were tested for normality, transformed when necessary and subjected to the PROC GLM (SAS 9.2 Institute, 2009). The tukey test was used to compare the means with the least square method and the significance level was detected at $p \leq 0.05$.

3 | RESULTS

3.1 | Fatty acids in soybean oil and tallow

The analyses of the major fatty acids in soybean oil and tallow are shown in Table 3. The ratio of SFAs to UFAs in tallow was 33:60 and in soybean oil was 16:84, indicating the presence of more SFAs in animal fat and consequently lower digestibility compared to vegetable oils.

3.2 | Growth performance

3.2.1 | Average BW and BWG

The effects of LPL, type of fat source and fat level in diet on average BW, BWG, feed consumption and feed conversion ratio during the 15

TABLE 2 Composition and nutrient content of diets^a (as-fed basis).

Diet ingredients	Soybean oil%		Tallow%		Soybean oil% + tallow% (50:50)	
	1.5	3	1.5	3	1.5	3
Corn	60.24	55.92	61.39	58.24	61.18	57.81
Soybean meal (44% CP)	33.29	34.15	33.06	33.69	33.10	33.78
Soybean oil	1.5	3.00	-	-	0.75	1.5
Tallow	-	-	1.5	3.00	0.75	1.5
Dicalcium phosphate	1.59	1.59	1.59	1.59	1.59	1.59
Limestone	0.94	0.93	0.94	0.94	0.94	0.94
Common salt	0.26	0.28	0.25	0.27	0.26	0.27
D,L-methionine	0.29	0.30	0.29	0.29	0.29	0.29
L-lysine hydrochloride	0.21	0.19	0.21	0.20	0.21	0.20
L-threonine	0.08	0.08	0.08	0.07	0.08	0.08
Vitamin and mineral premix ^b	0.50	0.50	0.50	0.50	0.50	0.50
NaHCO ₃	0.11	0.10	0.12	0.10	0.12	0.10
Choline chloride	0.07	0.07	0.07	0.07	0.07	0.07
Sand	0.92	2.89	-	1.04	0.16	1.37
Calculated composition%						
Metabolisable energy (kcal/kg)	2906.1	2906.1	2906.1	2906.1	2906.1	2906.1
Crude protein (%)	20.18	20.18	20.18	20.18	20.18	20.18
Calcium (%)	0.81	0.81	0.81	0.81	0.81	0.81
Available phosphorous (%)	0.40	0.40	0.40	0.40	0.40	0.40
Lysine (%)	1.20	1.20	1.20	1.20	1.20	1.20
Methionine (%)	0.60	0.60	0.60	0.60	0.60	0.60
Methionine + Cystine (%)	0.92	0.92	0.92	0.92	0.92	0.92
Threonine (%)	0.82	0.82	0.82	0.82	0.82	0.82
Tryptophan (%)	0.28	0.28	0.28	0.28	0.28	0.28
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15
Chlorine (%)	0.25	0.25	0.24	0.25	0.25	0.25
Potassium (%)	0.84	0.85	0.84	0.85	0.84	0.85
DCAD (mEq)	212.0	212.0	212.0	212.0	212.0	212.0

^aEach of the six diets was divided into two equal portions, and 0.025% lysophospholipid was added to the top of one portion and mixed well to make 12 diets in total.

^bAmount per kilogram diet: vitamin A (trans retinyl acetate), 12 500 International units; vitamin D₃ (cholecalciferol), 5000 International units; vitamin E (tocopheryl acetate), 80 mg; vitamin K₃, 3.2 mg; thiamine, 3.2 mg; riboflavin, 8.6 mg; pantothenic acid, 8.6 mg; pyridoxine, 4.86 mg; B₁₂ Cyanocobalamin, 0.02 mg; niacin, 62.51 mg; biotin, 0.25 mg; folic acid, 2.2 mg and antioxidant, 2.5 mg. Mineral premix supplied per kilogram diet: Fe, 20.23 mg; Mn, 120 mg; Zn, 110 mg; Cu, 16 mg; I, 1.252 mg; Se, 0.3 mg. Choline chloride, 300 mg (vitamin and mineral supplements).

to 28 days of the experiment are shown in Table 4. The main and interactive effects of LPL, type of fat source and fat level in diet were not significantly different on the average BW, BWG, FI and FCR in broiler chickens ($p < 0.05$).

3.2.2 | Feed conversion ratio

In our study, the FCR in broiler chickens was not affected by LPL supplementation, levels and sources of dietary fat during the grower period.

3.2.3 | Feed intake

This study shows that the addition of LPL, levels and source of dietary fat did not affect the FI of chickens during the growth period.

3.3 | Small intestinal morphology

The effect of LPL, levels and source of dietary fat on jejunum morphology in young broiler chickens is shown in Table 5. The jejunum villi width was significantly increased by the addition of 1.5% soybean oil and/or

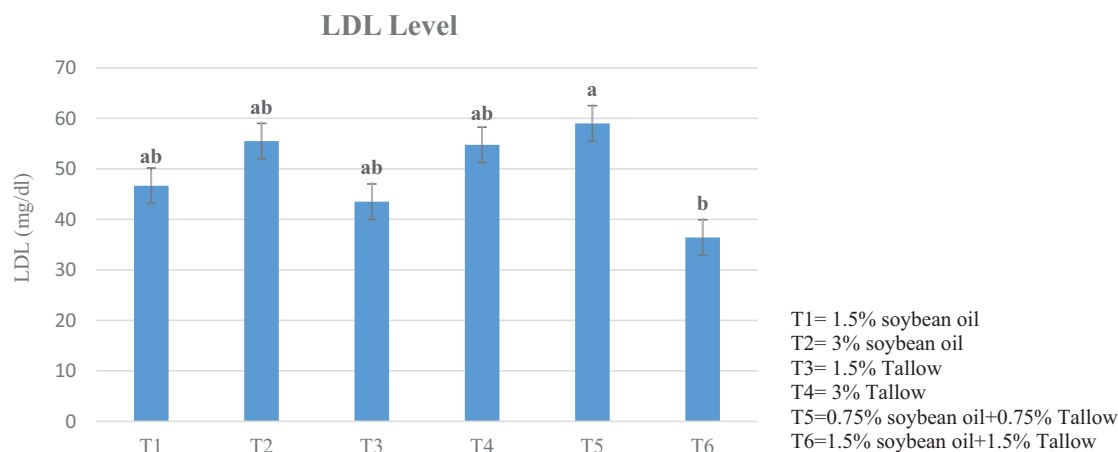


FIGURE 1 Interaction effects between source and level of fat in diet on serum LDL levels of broiler chickens at 28 days of age.

T1 = 1.5% soybean oil,
 T2 = 3% soybean oil,
 T3 = 1.5% tallow,
 T4 = 3% tallow,
 T5 = 0.75% soybean oil + 0.75% tallow,
 T6 = 1.5% soybean oil + 1.5% tallow.

TABLE 3 Analysis of the main fatty acids in tallow and soybean oil (%) used in this experiment.

Soybean oil	Tallow	Fatty acids ^a	
3.79	29.29	C18:0	Stearic acid
11.54	27.34	C16:0	Palmitic acid
0.5	3.04	C14:0	Lauric acid
23.51	28.51	C18:1	Oleic acid
52.78	3.81	C18:2	Linoleic acid
6.95	0.51	C18:3	Linolenic acid
16:84	60:33	SFA:UFA	

^aThe analyses are derived from the average of three replicates of each source of fat used.

tallow to the diet ($p < 0.05$). LPL supplementation of diet significantly increased crypt depth of the jejunum ($p < 0.05$).

3.4 | Serum lipid composition

The effect of LPL supplementation, levels and sources of dietary fat on the serum lipid composition in the broiler chickens at 28 days of age is shown in Table 6. The interaction effects of source and level of dietary fat on the serum LDL were significant ($p < 0.05$) and the 50/50 blend of soybean oil and tallow in the diet caused a significantly lower level of serum LDL when the mixture was used at 3% compared to 1.5% (Figure 1). The blood serum triglyceride level of broiler chickens showed a close-to-significant increase ($p = 0.057$) when birds were fed tallow. These findings may be attributed to the type and level of emulsifier and the composition of the diet. Other blood biochemical

parameters did not change in the present study, probably because of the low energy level of diets.

3.5 | Caeca microflora

The effect of LPL supplementation, source and levels of dietary fat on caeca microflora in broiler chickens at 28 days of age is shown in Table 7. The interactions between LPL supplementation, source and level of dietary fat had a significant effect on the population of *Lactobacilli* in caeca content ($p < 0.05$). LPL supplementation and a 3% blend of soybean oil and tallow (50:50) significantly improved the population of *Lactobacillus* bacteria (Figure 2).

4 | DISCUSSION

4.1 | Fatty acids in soybean oil and tallow

The presence of more SFAs reduces digestibility in animal fat compared to vegetable oils. Smits et al. (2000) reported that the 16:0 SFA from soybean oil has a higher absorption coefficient than the same (16:0) SFA in tallow, because the absorption of a fatty acid is related to the physicochemical properties of the type of fat or oil (Smits et al. 2000).

4.2 | Growth performance

4.2.1 | Average BW and BWG

Polin and Hussain's (1982) report was one of the first to show that the liver does not produce enough bile and the pancreas does not

TABLE 4 Effect of lysophospholipid supplementation, source and level of fat in the diet on broiler chickens performance (15–28 days of age).

Treatments	Average body weight (g/b)	Body weight gain (g/b)	Feed intake (g/b)	Feed conversion ratio (g/g)
Emulsifier lysophospholipid ^a				
+	1011.11	50.34	87.47	1.74
–	1013.18	50.74	85.73	1.7
SEM	8.65	0.6	1.23	0.02
Source of fat ^b				
Soybean oil	1020.54	51.12	87.77	1.75
Tallow	1002.67	50.45	86.31	1.73
Soybean oil + tallow (50:50)	1013.57	50.07	85.71	1.68
SEM	10.59	0.73	1.51	0.01
Fat diet ^c				
1.5	1022.52	51.17	86.68	1.7
3	1002	49.91	86.52	1.73
SEM	8.65	0.6	1.23	0.01
p-Value				
Emulsifier (A)	0.88	0.64	0.32	0.3
Fat source (B)	0.48	0.59	0.61	0.22
% (C) Fat diet	0.09	0.14	0.92	0.44
A × B	0.51	0.25	0.86	0.67
A × C	0.49	0.53	0.78	0.87
B × C	0.24	0.61	0.73	0.7
A × B × C	0.87	0.63	0.48	0.8

^aEach mean is the average of 36 observations.

^bEach mean is the average of 24 observations.

^cEach mean is the average of 36 observations.

SEM, standard error of mean.

actively secrete enough lipase and phospholipase enzymes during the early years of birds. They indicated that the addition of LPL in the diet had no effect on the BW of broilers, but it was assumed that with the increase in bird age and the increase in the level of LPL supplementation, there may be greater differences between the experimental treatments, through an increase in metabolic energy by LPL supplementation. In our study, the performance of broiler chickens was not improved by the addition of LPL to the diet. The investigators have shown that LPLs may increase nutrient digestibility and the apparent metabolisable energy of diets with reduced nutrient levels (Zhao et al., 2015). They observed that the digestibility of dry matter, gross energy, crude protein and crude fat was higher with the inclusion of 0.05% LPL in the diet compared to the control group in weaned pigs (difference in metabolic energy from the beginning to the end of the weaning period, 71.65 kcal/kg). Therefore, the possibility that broiler performance was not affected by emulsifier supplementation in our experiment can be attributed to the following factors: the insufficient level of added emulsifier to the diet, the low level of metabolic energy of the experimental diets (2906 kcal/kg) and the use of three experimental factors that caused a combined effect. Firman et al. (2008) reported that the addition of soybean oil or animal fats to diets did not have a significant effect on the final BW

of broilers. In the study of Huang et al. (2007), the addition of soybean oil to the diet compared to soybean lecithin increased the BW of broiler chickens grown up to 42 days of age. Whereas, in our study, there was no significant effect on the performance of birds when fed isocaloric and isonitrogenous diet that contained either tallow or soybean oil.

Sizemore and Siegel (1993) reported that increasing energy levels with constant protein decreased the BW of broilers in the starter period (1–21 days of age). The results of our experiment agree with the reports of many researchers (Ahmadi-Sefat et al., 2022; Zhao & Kim, 2017). Khonyoung et al. (2015) did not find any main or interaction effects of lysolecithin (0 and 145 mg/kg) and four types of fats on BWG. Zhao and Kim (2017) reported that the growth performance of broilers was not affected by the interaction of dietary energy levels and LPL (LPL) supplementation. Zampiga et al. (2016) showed that the LPL emulsifier (1.5 and 1 kg/ton) did not improve the final weight of broilers. These observations are almost in contrast with the research of Melegy et al. (2010), which showed the use of an emulsifier based on lysolecithin (0.25 or 0.5 kg/ton of feed) significantly improved the production characteristics of broiler chickens. Also, Ahmadi-Sefat et al. (2022) reported that LPLs supplementation (2 g/kg) had a positive effect on performance in broiler chickens fed with low-nutrient diets

TABLE 5 Effect of lysophospholipid supplementation, source and level of fat in diet on morphological variables of jejunum in broiler chickens on 28 days of age.

Treatments	Villus height (μm)	Willie width (μm)	Thickness of cover layer (μm)	Crypt depth (μm)	Villus height: Crypt depth
Emulsifier ^a lysophospholipid					
+	923.33	164.33	42.66	242.22 ^b	4.35
–	858.33	157.33	44.44	200.67 ^c	3.92
SEM	27.7	7.52	1.38	8.25	0.19
Source of fat ^d					
Soybean oil	873.5	179.33 ^b	44.33	228.5	3.95
Tallow	939.83	175.65 ^b	41.33	217.17	4.34
Soybean oil + tallow (50:50)	872.17	142.50 ^c	45	218.67	4.12
SEM	33.92	9.21	1.69	10.8	0.24
Fat diet ^e					
1.5	934.11	178.22 ^b	44.77	229.22	4.22
3	856.22	153.44 ^c	42.33	213.67	4.05
SEM	27.7	7.52	1.38	8.82	0.19
p-Value					
Emulsifier (A)	0.06	0.78	0.37	0.002	0.13
Fat source (B)	0.29	0.01	0.28	0.72	0.52
% Fat diet (C)	0.05	0.02	0.22	0.22	0.54
A × B	0.86	0.07	0.38	0.66	0.83
A × C	0.07	0.87	0.31	0.38	0.06
B × C	0.73	0.34	0.4	0.48	0.87
A × B × C	0.25	0.61	0.85	0.93	0.41

^aEach mean is the average of 36 observations.

^{b,c}Means in each column for many effects with uncommon superscripts are significantly different ($p < 0.05$).

^dEach mean is the average of 24 observations.

^eEach mean is the average of 36 observations.

SEM, standard error of mean.

(–100 Kcal/kg Metabolizable Energy (ME) and/or –5% Crude Protein (CP) and limiting amino acids) in the period of growth. Wu et al. (2022) reported that the growth performance of Jiangnan White goslings was improved by the hydroxylated lecithin concentration of 200 mg/kg. This contradiction may be due to the composition and level of phospholipids or the interaction between the type of fat and the emulsifier supplement in the diet. The UFA-rich sources are better utilised than SFAs in broilers (Leeson & Atteh, 1995; Smits et al., 2000). On the other hand, Reynier et al. (1985) showed that the micelle sizes and the durability of micelles are the most important factors in the absorption of lipids. The formation of smaller micelles may also influence the digestion of other nutrients.

Polycarpo et al. (2016) reported that the main effect of emulsifier supplementation positively influenced the performance, but not the interaction effects during the 1 to 42 days of age. Andreotti et al. (2004) and Ferreira et al. (2005) reported that there was no significant interaction between the emulsifier and increasing the fat levels in the diet on BW in broiler chicks. These results may be related to the addition of a high amount of fat along with an

incorrect level of emulsifier in the diet (Andreotti et al., 2004). Our findings are in agreement with others who reported that fat levels in isocaloric and isonitrogenous diets did not influence the BW of broiler chickens (Huang et al., 2007; Nobakht et al., 2011; Velasco et al., 2010).

4.2.2 | FCR

Wongsuthavas et al. (2007) and Nobakht et al. (2012) reported that the FCR was not affected by dietary fat levels. Wongsuthavas et al. (2007) reported that low lipase activity leads to impaired lipid digestion, resulting in very low energy being produced by increasing fat levels in isocaloric diet. In contrast, Velasco et al. (2010) reported that increased fat levels in diet improved the FCR of broilers throughout the growing period (1 to 34 days). Some differences in results may be attributed to the reduction of dust with high levels of fat in the diet, which may lead to improved fat efficiency as well as improved FCR (Rezaei et al., 2007).

TABLE 6 Effect of lysophospholipid supplementation, source and level of fat in diet on serum lipid composition in broiler chickens at 28 days of age.

Treatments	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Emulsifier lysophospholipid ^a				
+	105.44	138.11	70.61	46.5
–	105.66	141.94	68.61	52.11
SEM	7.69	7.32	4.44	3.93
Source of fat ^b				
Soybean oil	109.33 ^{c,d}	152.25	79.33	51.08
Tallow	116.83 ^c	136.58	64.16	49.12
Soybean oil +tallow (50: 50)	90.50 ^d	131.25	65.33	47.7
SEM	9.42	8.97	5.44	4.81
Fat diet% ^e				
1.5	100.11	135.72	65.94	49.72
3	111	144.33	73.27	48.88
SEM	7.69	7.32	4.44	3.93
p-Value				
Emulsifier (A)	0.97	0.71	0.75	0.31
Fat source (B)	0.057	0.24	0.11	0.88
Fat diet% (C)	0.22	0.41	0.25	0.88
A × B	0.35	0.46	0.53	0.6
A × C	0.95	0.56	0.19	0.68
B × C	0.81	0.12	0.67	0.02
A × B × C	0.1	0.34	0.81	0.3

^aEach mean is the average of 36 observations.

^bEach mean is the average of 24 observations.

^{c,d}Means in each column for many effect with uncommon superscript are significantly different ($p < 0.05$).

^eEach mean is the average of 36 observations.

SEM: standard error of mean.

Leeson and Summers (2001) reported that the absorption of fats and fatty acids differs in poultry, which can make a difference in their energy value. The main factor that influences the nutritional value of fat sources is digestibility, which is affected by the form of fat (triglycerides or fatty acids), fatty acid saturation degree, carbon number in the chain, ration composition, free fatty acid concentration, position of the glycerol molecules as well as the ratio of UFAs to SFAs in the mixture of free fatty acids, the number of fatty acid double bonds, the fatty acid chain length, intestinal microorganisms and the age of the bird (Leeson & Summers, 2001).

4.2.3 | Feed intake

Several studies reported that the inclusion of vegetable oil in high-energy diets resulted in improved FI (Monfaredi et al., 2011). Also FI may be increased by the high level of dietary fat, which reduces dust and improves palatability of the diet (Rezaei et al., 2007). Also, Polycarpo et al. (2016) reported that broilers fed diet containing soybean oil produced a higher BWG, while they had no significant effect on FI.

Similar to our results, Zhang et al. (2011) did not observe any differences on the performance by feeding casein, non-ionic and lysophosphatidyl choline emulsifiers. In our study, increasing dietary fat levels or emulsifier supplementation did not affect FI during the growth period. Also, Kusaibati et al. (1982) did not observe the effect of fat and emulsifier (lysolecithin 350 g/ton) on the FI of chickens in the whole experimental period. Contrary to our results, Abbas et al. (2016) reported an increase in FI and absorption of nutrients in broilers fed diets that contained 0.25 or 0.50 g/kg of lysolecithin.

4.3 | Small intestinal morphology

A greater villus height or crypt depth ratio is related to enhance nutrient absorption, and an increase in villi height is related to enhance epithelial turnover and cell mitosis (Yoon et al., 2012). Villus height and crypt depth values, as well as the villus height and crypt depth ratio, can be used to evaluate intestine health in broiler chickens (Xing et al., 2020). According to Nemati et al. (2021), de-oiled soybean lecithin

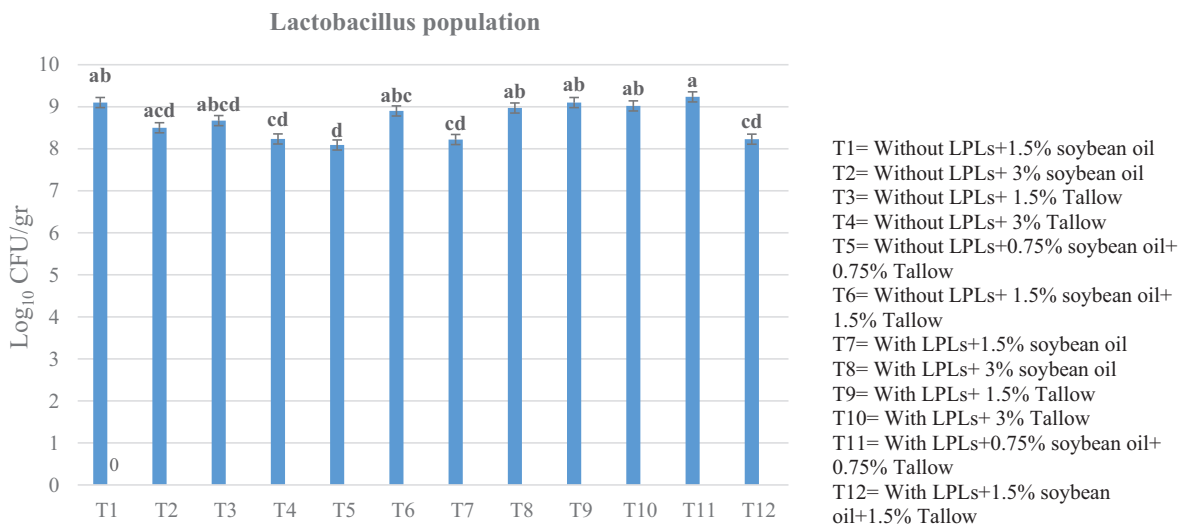


FIGURE 2 Interaction effects between lysophospholipid supplementation, source and level of fat in diet on the population of caeca *Lactobacillus* bacteria of broilers at 28 days of age.

T1 = Without LPLs + 1.5% soybean oil,
 T2 = Without LPLs + 3% soybean oil,
 T3 = Without LPLs + 1.5% tallow,
 T4 = Without LPLs + 3% tallow,
 T5 = Without LPLs + 0.75% soybean oil + 0.75% tallow,
 T6 = Without LPLs + 1.5% soybean oil + 1.5% tallow,
 T7 = With LPLs + 1.5% soybean oil,
 T8 = With LPLs + 3% soybean oil,
 T9 = With LPLs + 1.5% tallow,
 T10 = With LPLs + 3% tallow,
 T11 = With LPLs + 0.75% soybean oil + 0.75% tallow,
 T12 = With LPLs + 1.5% soybean oil + 1.5% tallow.

(1 and 2 g/kg) in the low-ME diet decreased the crypt depth in the turkey jejunum. Viñado et al. (2019) reported that the morphological parameters of the jejunum in broilers were not affected by dietary lecithin. These conflicting results may be due to the fact that previous studies used different levels and sources of fat and different emulsifiers (Viñado et al., 2019).

Boontiam et al. (2017) reported that the level of 0.05% LPL supplementation improved the villus height and crypt depth ratio and decreased duodenum and jejunum crypt depth. The results of Boontiam et al. (2017) showed that jejunum villus height was significantly increased in broiler chickens fed diets containing 0.05% LPL, which indicates an increase in the level of epithelial cells to absorb nutrients for optimal growth and production in broiler chickens. Khonyoung et al. (2015) observed the activated cell mitosis in the apical surface of villi in broilers fed diet with lysolecithin. The right mechanism of operation by which emulsifiers improve intestine morphology is not yet fully understood. It is possible that the emulsifier supplement, which acts by stimulating micelle synthesis in the gut, may improve the intestinal mucosa structure by reducing intestinal fermentation and, thus, minimising villous surface damage in broilers (Majdollahseini et al., 2019).

4.4 | Serum lipid composition

One of the markers of health conditions and malnutrition is serum lipid profile status in poultry (Boontiam et al., 2019). LPL supplementation or low-diet energy did not influence other serum biochemical characteristics in broiler chickens, which may be due to the age of the bird (28 days). Because, the activity of the liver, pancreas and other organs related to the secretion of enzymes involved in the metabolism of fat, calcium and phosphorus is evolved by the age of bird (28 days). Although, the LPL supplementation of diets in the present study did not modify the serum biochemical parameters, it may be related to the energy levels, which were lower than the standard diet. This is in consistent with the study of Wang et al. (2016), who found no difference in the concentration of serum triglycerides, total cholesterol, HDL and LDL with sodium stearoyl-2-lactylate supplementation of diets with lower energy levels in 35-day-old broiler chickens. Also, Neto et al. (2011) reported that emulsifier supplementation or dietary fat sources did not significantly affect total cholesterol, HDL and triglyceride levels in broiler chickens. Upadhaya et al. (2017) reported no significant difference ($p > 0.05$) in serum total cholesterol, triglyceride and HDL levels in broiler chickens fed diets containing emulsifier blend.

TABLE 7 Effect of lysophospholipid supplementation, source and level of fat in diet on caeca microflora in broiler chickens at 28 days of age.

Treatments	<i>Lactobacillus</i> (Log ₁₀ CFU/gr)	<i>E-Coli</i> (log ₁₀ CFU/gr)
Emulsifier lysophospholipid ^a		
+	8.89	4.94
–	8.58	4.89
SEM	0.09	0.15
Source of fat ^b		
Soybean oil	8.7	5.05
Tallow	8.75	5.09
Soybean oil + tallow (50: 50)	8.61	4.61
SEM	0.12	0.19
Fat diet% ^c		
1.5	8.73	5.05
3	8.64	4.78
SEM	0.09	0.14
p-Value		
Emulsifier (A)	0.12	0.83
Fat source (B)	0.7	0.17
Fat diet% (C)	0.49	0.24
A × B	0.06	0.4
A × C	0.91	0.95
B × C	0.61	0.22
A × B × C	0.0001	0.32

^aEach mean is the average of 36 observations.

^bEach mean is the average of 24 observations.

^cEach mean is the average of 36 observations.

SEM, standard error of mean.

In contrast, Fascina et al. (2009) reported that serum triglyceride levels were linearly decreased by increasing soybean oil in the diet. Roy et al. (2010) reported that the serum concentrations of LDL and total cholesterol were decreased by the emulsifier supplementation (glycerol polyethylene glycol ricinolate) when measured on the 20th day, but no significant difference was observed on the 39th day, which was in agreement with our results on the 28th day. This experiment revealed that feeding diets with low metabolisable energy levels with or without LPL supplementation would not alter the serum lipid profiles in broilers, thus, only a reduction of 150–200 kcal/kg of standard diet energy may help the health of the birds.

4.5 | Caeca microflora

Polycarpo et al. (2016) reported that broilers fed diet containing soybean oil had a lower total number of anaerobic bacteria in the jejunum. The normal level of bile salts and enzymes involved in the digestion of lipids may not be sufficient to effectively influence the

emulsification of fatty acids in tallow, large parts of which are completely SFAs. The correlation between the low digestibility of saturated fat and intestinal microflora was reported by Danicke et al. (1999). The integrity of bile salts through the activity of bacterial taurine hydrolase was affected by the increase in the level of microbial activity (Knarreborg et al., 2002), which affects the digestibility and performance of chickens (Feighner & Dashkevich, 1987). In addition, Maisonnier et al. (2003) showed that the loss of bile salts is caused by intestinal microflora. Therefore, the positive effects of LPL in diets containing tallow may also be related to the anti-microbial effect by improving fat digestibility.

5 | CONCLUSIONS

This experiment revealed that feeding diets with a low metabolisable energy level (around 2900 Kcal/kg) with or without LPL supplementation does not change performance, small intestinal morphology and serum lipid composition in broiler chickens, but may cause an increase in the population of caeca *lactobacilli*. Thus, a reduction of 150–200 kcal/kg in recommended dietary energy with LPL supplementation may help in the health of broilers through an increase in caeca *lactobacilli*.

AUTHOR CONTRIBUTIONS

Aliakbar Salari: conceptualisation; data curation; formal analysis; investigation; methodology; project administration; resources; software; writing—original draft; writing—review & editing; visualisation.

Abolghasem Golian: correspondence; conceptualisation; funding acquisition; methodology; supervision; validation.

Ahmad Hassanabadi: conceptualisation & editing; supervision; validation.

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CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the author(s).

ETHICS STATEMENT

All procedures were approved by the Animal Care and Use Committee of the Ferdowsi University of Mashhad, Mashhad, Iran.

AUTHOR DECLARATIONS

All authors are either employed by, or associated with, a government agency or university, whose primary function is research and education.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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