



RESEARCH ARTICLE

Effects of Lysolecithin Supplementation as an Emulsifier in Broiler Diets on Growth Performance, Serum Biochemical Parameters and Nutrient Digestibility

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Abstract

Objective: This study evaluated the effects of lysolecithin (LL) supplementation in diets containing two lipid sources (LSs) and two levels of Metabolisable Energy (ME) on growth performance, slaughter characteristics, serum biochemical parameters and nutrient digestibility in broilers.

Materials and Methods: A total of 640 one-day-old male broiler chicks were randomly allocated to eight experimental treatments in a 2×2×2 factorial arrangements. The experimental factors included two ME levels, namely, a normal-ME diet containing the commercially recommended ME level and a low-ME diet containing 100 kcal/kg less ME than the commercial recommendation; two LSs, soybean oil (SO) and turkey fat (TF); and two levels of dietary LL supplementation, 0 and 1 kg/ton.

Results: During the overall experimental period, diets containing SO improved broiler performance compared with diets containing TF ($p<0.05$). Similarly, the normal-ME diet improved final body weight and body weight gain ($p<0.01$), feed intake ($p<0.05$) and feed conversion ratio (FCR; $p<0.001$) compared with the low-ME diet. Dietary LL supplementation also improved overall growth performance ($p<0.05$). Supplementation with LL increased serum high-density lipoprotein (HDL) cholesterol concentration ($p<0.05$) and crude protein digestibility ($p<0.05$) when added to SO-containing diets, whereas these responses decreased when LL was added to TF-containing diets ($p<0.05$). Furthermore, LL supplementation increased crude fat digestibility in diets containing TF ($p<0.05$).

Conclusion: The effects of LL supplementation on broiler performance and nutrient utilization were influenced by the dietary lipid source and ME level. Overall, SO and normal dietary ME levels supported better broiler performance, while LL supplementation improved performance and nutrient digestibility, with some responses differing according to the lipid source.

INTRODUCTION

World population is predicted to increase by more than one-third, reaching over 9 billion people by the year 2050. In this respect, the expected increase in broiler meat

consumption as a source of animal protein from 2010 to 2050 is estimated to be 173%¹. Broiler meat is preferred over other meat products and is available throughout the year at relatively lower prices than beef, mutton, and fish. In recent years, continuous genetic improvements in commercial strains

have shortened the production cycle of broiler chickens and increased their growth rate, leading to greater nutritional demands and energy requirements. In addition to cereal grains, the inclusion of lipid sources, such as vegetable oils and animal fats, in broiler diets is one of the most common strategies for meeting the high energy requirements of fast-growing broilers because of their high energy content². However, increasing the inclusion levels of lipid sources, such as vegetable oils and animal fats, in broiler diets can result in the incomplete utilization of dietary lipids by broilers and increased feed and production costs, as lipid sources are relatively expensive³. Lipid digestibility and utilization are significantly more complex than those of other macronutrients and are largely dependent on the age and genetics of broilers, the secretion and activity of lipase and bile salts in the digestive system and the source and fatty acid profile of dietary lipid sources⁴. The digestibility and absorption of oils rich in unsaturated fatty acids (UFAs) are higher than those of fats rich in saturated fatty acids (SFAs)². Furthermore, lipids are insoluble in water, do not readily dissolve in the aqueous environment of the gastrointestinal tract, and require emulsification before they can be effectively hydrolyzed by lipase⁵. The source, fatty acid profile and physical form of dietary lipids may influence the effectiveness of emulsifiers in lipid digestion⁶. Exogenous emulsifiers appear to be more effective in chickens fed diets supplemented with soybean oil (rich in UFAs) than in those fed diets supplemented with palm oil (saturated fat)⁷.

In view of the above-mentioned factors, further studies are needed to develop innovative dietary strategies that can effectively reduce the high costs of feed in broiler production by minimizing Metabolizable Energy (ME) intake while ensuring optimal performance and health of broilers^{8,9}. Improving lipid digestibility and utilization may enable a reduction in the inclusion levels of supplemental lipids in broiler diets, thereby lowering dietary ME and feed production costs without impairing broiler performance⁹. In this context, the inclusion of emulsifiers, such as lysophospholipids and lysolecithins, in broiler diets is considered an effective strategy to improve the digestibility of lipid sources and reduce dietary ME content without adversely affecting the growth performance of broilers^{2,9}. Exogenous emulsifiers in broiler diets function as molecular surfactants with both hydrophobic and hydrophilic properties, thereby improving the dispersion of oil droplets in oil-water emulsions. This process increases the surface area of lipids and facilitates the activity of lipase enzymes. These lipase enzymes break down triglyceride molecules into free fatty acids and monoglycerides, facilitating

the formation of micelles containing lipolytic products, which increases lipid digestion and absorption in the small intestine of broilers and enhances the availability of ME in their diets¹⁰.

However, the hydrophilic-lipophilic balance (HLB) is a key indicator for the selection of an appropriate emulsifier. Emulsifiers with low HLB numbers are soluble in oil, whereas emulsifiers with high HLB numbers are soluble in the aqueous environment of the small intestine. Since birds consume 1.5-2 times more water than feed, only a small amount of fat is present in the diet, and the amount of water in their digestive system should be greater than the amount of fat. Therefore, the use of exogenous emulsifiers with high HLB numbers, such as lysolecithins, in poultry diets is recommended¹¹. Several studies have shown that lysolecithin supplementation in broiler diets enhances micelle and emulsion formation by supporting bile salt activity⁹. Therefore, emulsifier supplementation is essential for lipid digestibility and absorption in broilers. Accordingly, many studies have demonstrated that dietary lysolecithin supplementation improves lipid availability and overall performance in broilers^{8,9}, enhances ME availability and lipid digestibility^{8,12,13}, improves serum lipid profiles^{13,14}, and decreases abdominal fat deposition¹⁴. Consequently, emulsifier supplementation is considered an important nutritional strategy for improving lipid digestibility and absorption in broilers.

Many factors, including the source, fatty acid profile and physical form of lipids, may affect their utilization by broilers. It has been reported that the digestibility and absorption rates of UFAs in vegetable oils, such as soybean oil, canola oil, and corn oil, are higher than those of SFAs in animal fats, such as poultry fat, beef tallow and lard². Exogenous emulsifiers appear to be more effective in chickens fed diets containing soybean oil (SO), which is rich in polyunsaturated FAs, than in those fed diets supplemented with palm oil, a saturated fat⁷. Zhang *et al.*¹⁵ pointed out that broilers fed diets containing poultry fat (PF) had similar growth performance to those fed diets containing SO, suggesting that poultry fat is a suitable fat source for broilers. Contrary to this finding, Oliveira *et al.*² reported that dietary lipid source (soybean oil or chicken fat) had no significant effect on the growth performance of broilers from 1 to 42 days of age. Therefore, the source, fatty acid profile, and physical form of dietary lipids may influence the effectiveness of dietary emulsifiers in lipid digestion⁶.

Recent studies have investigated the effects of emulsifiers on enhancing ME availability in poultry diets. The use of exogenous emulsifiers in low-ME diets has been shown to improve growth performance, nutrient and apparent metabolizable energy (AME) digestibility, and carcass yield in broilers^{2,16,17}. Moreover, Jansen *et al.*¹⁸ reported that the addition of lysolecithin to broiler diets improved dry matter

digestibility, nitrogen retention, and AMEn, particularly when saturated fat sources were incorporated. Some previous studies have suggested that adding emulsifiers to low-ME diets containing the same oil or fat sources^{10,19,20} improves growth performance and the digestibility of lipids and AME. In contrast to the aforementioned studies, some studies have also reported that emulsifier supplementation in normal- and low-energy-density diets containing similar lipid sources had no significant effect on the growth performance or nutrient digestibility of broilers^{21,22}. Furthermore, Ge *et al.*²³ reported that high- and low-energy diets had no significant effects on serum biochemical parameters in broiler chickens.

However, to our knowledge, limited and inconsistent information is available in the literature^{5,24} regarding the effects of emulsifier supplementation in diets with different ME levels and containing different lipid sources on the growth performance, nutrient digestibility and serum biochemical profile of broilers. Consequently, the objective of the present study was to evaluate the effects of lysolecithin supplementation in diets containing different lipid sources and having different ME levels on growth performance, serum biochemical profile, slaughtering parameters and nutrient digestibility in broilers.

MATERIALS AND METHODS

Animal care: The complete experimental protocol was reviewed and approved by the Animal Care and Use Committee of Tokat Gaziosmanpasa University (Process No. 2019-HADYEK-45).

Animals, diets and experimental design: On the day of hatching, a total of 640 one-day-old male Ross 308 broiler chicks were obtained from a commercial hatchery (Garanti Chicken Farming, Bolu, Türkiye). The chicks were weighed, wing-banded, and randomly distributed into eight treatment groups, with four replicates of 20 chicks per replicate, ensuring similar initial mean body weights among treatment groups. From hatching to 42 days of age, the chicks were housed in floor pens bedded with fresh wood shavings. The temperature was maintained at 32°C during the first week, reduced to 28°C during the second week and gradually decreased thereafter, reaching 21°C after 27 days of age. A fluorescent lighting schedule of 23 hrs light and 1 hr dark was maintained throughout the experiment, with an average light intensity of 20 lux. The experimental diets were provided in mash form, and drinking water was provided *ad libitum*.

The experimental treatments were arranged in a 2×2×2 factorial design comprising two ME levels, namely, a normal-

ME diet containing the commercially recommended ME level and a low-ME diet containing 100 kcal/kg less ME than the commercial recommendation; two lipid sources, soybean oil (SO) and turkey fat (TF) and two levels of lysolecithin supplementation, LL0 and LL1, corresponding to 0 and 1 kg/t, respectively. Lysolecithin (Lysoforte; LL), used as the emulsifier source in the experimental diets, was obtained from Kemin Chemical Products Marketing and Foreign Trade Ltd. Co. (Istanbul, Türkiye). Feed-grade SO and TF were purchased from a commercial company producing SO and TF.

Prior to experimental diet formulation, the feed ingredients were analyzed for dry matter, crude protein (CP), ether extract, crude ash, starch, and total sugar contents according to the methods of the AOAC²⁵ at the Ankara Food Control Laboratory (Ankara, Türkiye). The ME contents of SO and TF used in the diets were analyzed by Kemin Chemical Products Marketing and Foreign Trade Ltd. Co. All diets were formulated based on the analyzed nutrient composition of the feed ingredients and according to phase-feeding practices as the broiler chickens advanced in age and body weight, following the recommendations of the Ross 308 breeder²⁶. The starter phase lasted from day 0 to 10, the grower phase from day 11 to 28 and the finisher phase from day 29 to 42. The ingredient and nutrient compositions of the experimental diets used during the different growth periods are presented in Table 1-3.

Growth performance: During the 42-day experimental period, broiler growth performance was evaluated based on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). The body weight (BW) of the broilers was recorded at the beginning of the experiment and weekly thereafter. Weekly BWG was calculated by subtracting the previous BW from the current BW and dividing the resulting value by the number of broilers in each replicate. Weekly FI was calculated by subtracting the weight of the feed remaining in the feeders from the weight of feed provided during the corresponding week and dividing the resulting value by the number of broilers in each replicate. Mortality was recorded and considered when calculating FI. The FCR was calculated weekly by dividing the average FI by the average BWG of the broilers.

Nutrient digestibility: On day 36 of age, eight broilers per treatment group were individually transferred to cleaned metabolism cages. Nutrient digestibility was determined using chromium oxide as an indigestible marker. The broilers were fed the experimental diets containing 3 g chromium oxide/kg from day 36 to 42 to assess nutrient digestibility. The broilers

Table 1: The ingredients and nutrient composition of the experimental diets used during the starter period

Ingredients	NMESOLLO	NMESOLL1	NMETFLL0	NMETFLL1	LMESOLLO	LMESOLL1	LMETFLL0	LMETFLL1
Soybean oil	2.00	2.00	-	-	0.50	0.50	-	-
Turkey fat	-	-	2.00	2.00	-	-	0.50	0.50
Corn	57.27	57.27	57.44	57.44	58.19	58.19	58.35	58.35
Fish meal	1.46	1.46	1.72	1.72	0.42	0.42	0.42	0.42
Soybean meal	34.86	34.86	34.45	34.45	36.35	36.35	36.19	36.19
DCP	2.20	2.20	2.19	2.19	2.26	2.26	2.26	2.26
DL-methionine	0.36	0.36	0.36	0.36	0.37	0.37	0.37	0.37
Limestone	0.87	0.87	0.86	0.86	0.90	0.90	0.90	0.90
L-lysine	0.22	0.22	0.22	0.22	0.24	0.24	0.24	0.24
L-threonine	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Salt	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.35
Vitamin premix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix ²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Dry matter (%)	90.10	90.10	90.10	90.10	89.90	89.90	89.90	89.90
Crude protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
ME (kcal/kg)	3025.00	3025.00	3025.00	3025.00	2925.00	2925.00	2925.00	2925.00
Ca (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P available (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Met+Sis (%)	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Lysine (%)	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44
Threonine (%)	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Tryptophan (%)	0.31	0.31	0.30	0.30	0.31	0.31	0.31	0.31
Lysolecithin (LL) (%)	-	0.10	-	0.10	-	0.10	-	0.10

¹Vitamin premix/2.5 g/kg diet: 15 000 IU Vitamin A, 3 000 IU vitamin D₃, 50 mg vitamin E, 5 mg vitamin K₃, 3 mg vitamin B₁, 6 mg vitamin B₂, 5 mg vitamin B₆, 0.03 mg vitamin B₁₂, 25 mg niacin, 12 mg Ca-D-pantothenate, 1 mg folic acid, 0.05 mg D-biotin, 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride ²Trace Mineral Premix/kg diet: 80 mg Mn, 60 mg Fe, 60 mg Zn, 5 mg Cu, 0.2 mg Co, 1 mg I, 0.15 mg Se, NMESOLLO: Diet including normal ME and SO and without LL, NMESOLL1: Diet including normal ME and SO and supplemental LL, LMESOLLO: Diet including low ME and SO and without LL, LMESOLL1: Diet including low ME and SO and supplemental LL; NMETFLL0: Diet including normal ME and TF and without LL; NMETFLL1: Diet including normal ME and TF and supplemental LL; LMETFLL0: Diet including low ME and TF and without LL; LMETFLL1: Diet including low ME and TF and supplemental LL

Table 2: The ingredients and nutrient composition of the experimental diets used during the grower period

Ingredients	NMESOLLO	NMESOLL1	NMETFLL0	NMETFLL1	LMESOLLO	LMESOLL1	LMETFLL0	LMETFLL1
Soybean oil	3.50	3.50	-	-	1.80	1.80	-	-
Turkey fat	-	-	3.50	3.50	-	-	1.80	1.80
Corn	58.84	58.84	59.14	59.14	60.51	60.51	60.76	60.76
Fish meal	2.65	2.65	3.10	3.10	2.15	2.15	2.40	2.40
Soybean meal	31.49	31.49	30.77	30.77	31.99	31.99	31.50	31.50
DCP	1.85	1.85	1.83	1.83	1.88	1.88	1.87	1.87
DL-methionine	0.22	0.22	0.21	0.21	0.22	0.22	0.22	0.22
Limestone	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Salt	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Vitamin premix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Mineral premix ²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Dry matter (%)	90.10	90.10	90.10	90.10	90.00	90.00	90.00	90.00
Crude protein (%)	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
ME (kcal/kg)	3150.00	3150.00	3150.00	3150.00	3050.00	3050.00	3050.00	3050.00
Ca (%)	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
P available (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Met+Sis (%)	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Lysine (%)	1.23	1.23	1.23	1.23	1.22	1.22	1.22	1.22
Threonine (%)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Tryptophan (%)	0.29	0.29	0.29	0.29	0.28	0.28	0.28	0.28
Lysolecithin (LL) (%)	-	0.10	-	0.10	-	0.10	-	0.10

¹Vitamin premix/2.5 g/kg diet: 15 000 IU Vitamin A, 3 000 IU vitamin D₃, 50 mg vitamin E, 5 mg vitamin K₃, 3 mg vitamin B₁, 6 mg vitamin B₂, 5 mg vitamin B₆, 0.03 mg vitamin B₁₂, 25 mg niacin, 12 mg Ca-D-pantothenate, 1 mg folic acid, 0.05 mg D-biotin, 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride, ²Trace Mineral Premix/kg diet: 80 mg Mn; 60 mg Fe; 60 mg Zn; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se, NMESOLLO: Diet including normal ME and SO and without LL; NMESOLL1: Diet including normal ME and SO and supplemental LL; LMESOLLO: Diet including low ME and SO and without LL; LMESOLL1: Diet including low ME and SO and supplemental LL; NMETFLL0: Diet including normal ME and TF and without LL; NMETFLL1: Diet including normal ME and TF and supplemental LL; LMETFLL0: Diet including low ME and TF and without LL; LMETFLL1: Diet including low ME and TF and supplemental LL

Table 3: The ingredients and nutrient composition of the experimental diets used during the finisher period

Ingredients	NMESOLLO	NMESOLL1	NMETFLL0	NMETFLL1	LMESOLLO	LMESOLL1	LMETFLL0	LMETFLL1
Soybean oil	4.12	4.12	-	-	2.25	2.25	-	-
Turkey fat	-	-	4.12	4.12	-	-	2.25	2.25
Corn	63.70	63.70	63.84	63.84	65.97	65.97	65.91	65.91
Soybean meal	28.39	28.39	28.25	28.25	28.00	28.00	28.06	28.06
DCP	2.10	2.10	2.10	2.10	2.10	2.10	2.10	2.10
DL-methionine	0.18	0.18	0.18	0.18	0.17	0.17	0.17	0.17
Limestone	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Vitamin premix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix ²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Dry matter (%)	90.10	90.10	90.10	90.10	89.90	89.90	89.90	89.90
Crude protein (%)	19.00	19.00	19.00	19.00	19.00	19.00	19.00	19.00
ME (Kcal/kg)	3200.00	3200.00	3200.00	3200.00	3100.00	3100.00	3100.00	3100.00
Ca (%)	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
P available (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Met+Sis (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Lysine (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Threonine (%)	0.71	0.71	0.71	0.71	0.72	0.72	0.72	0.72
Tryptophan (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysolecithin (LL) (%)	-	0.10	-	0.10	-	0.10	-	0.10

¹Vitamin premix/2.5 g/kg diet: 15 000 IU Vitamin A, 3 000 IU vitamin D₃, 50 mg vitamin E, 5 mg vitamin K₃, 3 mg vitamin B₁, 6 mg vitamin B₂, 5 mg vitamin B₆, 0.03 mg vitamin B₁₂, 25 mg niacin, 12 mg Ca-D-pantothenate, 1 mg folic acid, 0.05 mg D-biotin, 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride, ²Trace Mineral Premix/kg diet: 80 mg Mn; 60 mg Fe; 60 mg Zn; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se, NMESOLLO: Diet including normal ME and SO and without LL; NMESOLL1: Diet including normal ME and SO and supplemental LL; LMESOLLO: Diet including low ME and SO and without LL; LMESOLL1: Diet including low ME and SO and supplemental LL; NMETFLL0: Diet including normal ME and TF and without LL; NMETFLL1: Diet including normal ME and TF and supplemental LL; LMETFLL0: Diet including low ME and TF and without LL; LMETFLL1: Diet including low ME and TF and supplemental LL

were adapted to the diets containing chromium oxide for three consecutive days, from day 36 to 39. During the final 3 days, from day 39 to 42, fecal excreta samples were collected. The collected fecal excreta samples were dried in an oven at 55 °C for 24 hrs. The dried fecal excreta samples and experimental diets were ground using a laboratory mill and analyzed for dry matter (DM), crude protein (CP), and crude fat (CF) according to the method described by AOAC²⁵. The chromium oxide contents of the dried fecal excreta samples and experimental diets were determined using the method of Fenton and Fenton²⁷. The apparent digestibility of DM, CP, and CF was calculated using the following equation outlined by Jalal *et al.*²⁰:

$$\text{Apparent digestibility (AD) (\%)} = \left[1 - \frac{\left(\frac{\text{Cr}_2\text{O}_3 \text{ in diet}}{\text{Cr}_2\text{O}_3 \text{ in fecal excreta}} \right)}{\left(\frac{\text{Nutrient in diet}}{\text{Nutrient in fecal excreta}} \right)} \right] \times 100$$

Slaughtering parameters: At the end of the experiment, feed was withdrawn 6 hrs before slaughter²⁸. After the 6 hrs feed withdrawal period, two broilers with BWs similar to the mean BW of their respective treatment group were selected from each replicate, resulting in eight broilers per treatment and a total of 64 broilers for slaughter. The broilers were slaughtered

by severing the jugular vein after administration of sodium pentobarbital (100 mg/kg) as anesthesia. The carcasses were immediately plucked, processed by removing the head and feet, eviscerated by removing the gastrointestinal tract, weighed, and subsequently chilled overnight in a refrigerator at +4 °C. The measurements included pre-slaughter BW, hot and cold carcass weights, and the weights of selected internal organs. The heart, liver, gizzard, proventriculus, spleen, pancreas, small intestine, gall bladder, and abdominal fat were individually weighed to the nearest ± 0.001 g. The weights of the internal organs and hot and cold carcass yields were expressed as percentages of the pre-slaughter BW of the broilers.

Serum biochemical parameters: At the end of the experiment, on day 42, blood samples were collected from the 64 broilers slaughtered by severing the jugular vein, with eight broilers sampled from each treatment group. Blood samples were collected into tubes without anticoagulant. Serum was separated by centrifugation at 3500 rpm for 15 min at 4 °C. The separated serum was immediately stored at -80 °C until analysis of the serum biochemical parameters. Serum total cholesterol (TC), HDL and VLDL cholesterol, triglyceride (TG), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were determined using Autoanalyser test kits (Audit Diagnostics, Ireland) with an

Audit Autoanalyser (Autolab, AMS Srl, The Netherlands), according to the manufacturers' instructions. Serum biochemical parameters were analyzed at the Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuzmayis University.

Statistical analysis: The Kolmogorov-Smirnov and Shapiro-Wilk tests were initially used to assess the normality of the data, and a test for homogeneity of variance was also performed. All data obtained in the present study showed a normal distribution. The collected data were analyzed using ANOVA for a $2 \times 2 \times 2$ factorial design with the General Linear Models procedure of the SPSS (17.0)[®] statistical package²⁹. The statistical model included the effects of ME level, lipid source, lysolecithin level, and their interactions. Significant differences among treatment means were determined using Duncan's multiple range test³⁰. All statements of statistical significance were based on a $p < 0.05$.

RESULTS AND DISCUSSION

Growth performance: The effects of the experimental treatments on the final BW, BWG, FI, and FCR of broilers from hatching to 42 days of age are shown in Table 4. Broiler

chickens fed SO-containing diets had higher final BW ($p < 0.05$), BWG ($p < 0.05$) and FI ($p < 0.05$) and lower FCR ($p < 0.05$) than those of broilers fed TF-containing diets during the period from 0 to 42 days (Table 4).

Our results related to BWG and FCR are consistent with the findings reported by Fascina *et al.*³¹ and Zaefarian *et al.*³², who showed that feeding vegetable oil (SO)-containing diets significantly increased BWG and improved FCR in broilers compared with those fed animal fat (tallow)-containing diets. Likewise, Shoaib *et al.*¹² found that the use of SO in the diet significantly improved FCR in broilers compared with PF. In the present study, the positive effects of SO-containing diets on BW, BWG, and FCR of broilers are likely attributable to the higher digestibility of UFAs in SO and their more favorable contribution to meeting the energy requirements of the body than SFAs in TF³³. In fact, SO, which is rich in UFAs, is more readily emulsified by native bile salts because of its greater potential for micelle formation in the small intestine than TF, which is rich in SFAs. This may increase lipid and metabolisable energy bioavailability and ultimately result in greater BW and BWG and improved FCR in broilers¹². Our results for final BW, FI, and FCR during the overall experimental period disagree with the findings of Majdolhosseini *et al.*²⁴ and Oliveira *et al.*², who showed that there were no significant

Table 4: Effects of the experimental treatments on the final BW and BWG, FI and FCR of broilers from hatching to 42 days¹

Lipid source	ME Level	Lysolecithin (LL) level	Initial BW	Final BW	BWG	FI	FCR
Soybean oil (SO)	Normal	0 kg/t	40.41	2869.91	2829.50	4547.05	1.61
		1 kg/t	40.37	2885.42	2845.05	4565.92	1.60
Soybean oil (SO)	Low	0 kg/t	40.26	2672.50	2632.24	4684.53	1.78
		1 kg/t	40.22	2763.56	2723.34	4714.82	1.73
Turkey fat (TF)	Normal	0 kg/t	40.23	2736.73	2696.50	4491.73	1.67
		1 kg/t	40.38	2809.18	2768.80	4587.85	1.66
Turkey fat (TF)	Low	0 kg/t	40.26	2650.77	2610.51	4576.36	1.75
		1 kg/t	40.38	2758.80	2718.42	4680.00	1.72
SEM			0.244	21.790	21.675	24.863	0.014
Lipid source (LS)							
SO			40.32	2797.77 ^a	2757.46 ^a	4628.08 ^a	1.68 ^b
TF			40.31	2738.87 ^b	2698.55 ^b	4583.99 ^b	1.70 ^a
SEM			0.391	26.592	26.361	32.625	0.014
ME level (MEL)							
Normal			40.35	2825.31 ^a	2784.96 ^a	4548.14 ^b	1.63 ^b
Low			40.28	2711.33 ^b	2671.05 ^b	4663.93 ^a	1.75 ^a
SEM			0.391	26.592	26.361	32.625	0.014
Lysolecithin (LL) Level (kg/ton)							
0 (LL0)			40.29	2732.40 ^b	2692.11 ^b	4574.92 ^b	1.70 ^a
1 (LL1)			40.34	2804.24 ^a	2763.90 ^a	4637.15 ^a	1.68 ^b
SEM			0.391	26.592	26.361	32.625	0.014
p-value							
LS			0.998	0.013	0.013	0.034	0.038
MEL			0.904	0.006	0.005	0.020	0.000
LL			0.935	0.021	0.021	0.046	0.035
LS × MEL Interaction			0.882	0.235	0.232	0.576	0.073
MEL × LL Interaction			0.989	0.466	0.462	0.607	0.167
LS × LL Interaction			0.873	0.629	0.628	0.849	0.648
LS × MEL × LL Interaction			0.989	0.791	0.790	0.553	0.435

^{a,b} Values in the same column not sharing a common superscript differ significantly (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), SEM: Standard Error of the Mean and ¹ Data are means of four replicates per treatment group

differences between broilers fed diets containing SO or PF in terms of final BW, FI, and FCR during the period from 0 to 42 days.

As indicated in Table 4, feeding low-ME diets decreased final BW ($p < 0.01$) and BWG ($p < 0.01$), increased FI ($p < 0.05$) and deteriorated FCR ($p < 0.001$) in broiler chickens compared with those fed normal-ME diets during the 0-6-week period. Our results regarding growth performance are in agreement with the findings of Kamel *et al.*⁶, Ahmadi-Sefat *et al.*³⁴ and Jalal *et al.*²⁰, who showed that feeding reduced-ME diets decreased final BW and BWG, increased FI, and deteriorated FCR in broilers during the overall experimental period.

The dietary energy was initially utilized by rapidly growing broilers for maintenance and growth. Therefore, growth performance parameters, such as BW and BWG, might be reduced when the energy requirements of broilers are not adequately met for maintenance and growth, and nutrient digestibility decreases because of the reduced dietary ME content³⁵. Moreover, broilers consuming low-ME diets increase their FI to meet their energy requirements, consequently resulting in an increase in FCR²⁰. Furthermore, LL1 diets increased final BW ($p < 0.05$) and BWG ($p < 0.05$) and FI ($p < 0.05$) and improved FCR ($p < 0.05$) in broilers from 0 to 42 days compared with those of chickens fed LL0 diets (Table 4). These findings are consistent with previous reports of increased BW^{2,6,8,34}, BWG^{6,8,34} and FI² and improved FCR^{6,21,22,24,34} in broilers fed diets supplemented with emulsifiers. In our study, dietary supplementation with LL as an emulsifier improved the growth performance of broilers by enhancing fat digestion and absorption and consequently, energy utilization⁸. In addition, dietary emulsifiers may improve the adsorption-desorption balance, which is influenced by amphiphilic molecules such as fats, phospholipids and proteins. The changes induced by exogenous emulsifiers may improve nutrient absorption across the enterocyte membrane, thereby enhancing the bioavailability of nutrients in the diet. Consequently, the improvement in growth performance of broilers following dietary LL supplementation may be attributed to the increased digestibility of crude fat and crude protein observed in the present study^{8,9}. Conversely, several studies have revealed that BW^{22,24}, BWG^{21,22,24}, FI^{8,21,24,34} and FCR^{2,8} of broilers were not affected by dietary emulsifier supplementation compared with diets without emulsifier supplementation. The discrepancy in growth performance results may be attributed to differences in the levels and types of emulsifiers supplemented to the diets in previous studies²⁰. The two-way interaction effects of LS×MEL, MEL×LL and LS×LL, as well as the three-way interaction effect of

LS×MEL×LL, on final BW, BWG, FI, and FCR of broilers during the overall experimental period (0-42 days) were not significant (Table 4).

Slaughtering parameters: The effects of the experimental treatments on carcass yields and the relative weights (RW) of selected internal organs of broilers at 42 days of age are presented in Table 5. Neither the main effects of LS, ME level, and LL nor their two-way interactions, LS×MEL, MEL×LL and LS×LL, or the three-way interaction, LS×MEL×LL, significantly affected hot and cold carcass yields or the RW of the heart, gizzard, spleen, bile duct, and proventriculus in broilers (Table 5).

The SO-containing diet decreased the RW of abdominal fat ($p < 0.05$) and increased the RW of the liver ($p < 0.01$) in broilers compared with those fed the TF-containing diet (Table 5). These results agree with those of Majdolhosseini *et al.*²⁴, who reported that SO-containing diets resulted in lower abdominal fat deposition in broilers compared with PF-containing diets. This reduction may be associated with the fact that energy from SO, which is rich in PUFA, is more readily utilized for metabolic purposes than energy from PF, which is rich in SFA, thereby reducing body fat, particularly abdominal fat, accumulation²⁴.

In contrast to these findings, several studies have reported that the RW of abdominal fat^{36,37} and liver^{24,37} in broilers were not affected by dietary lipid sources. The discrepancy in the results regarding the RW of abdominal fat and liver may be attributable to differences in the amount of LS incorporated into the broiler diets. Feeding the normal-ME diet increased the RW of abdominal fat ($p < 0.05$) and liver ($p < 0.01$) and decreased the RW of the small intestine ($p < 0.05$) and pancreas ($p < 0.01$) in broilers (Table 5) compared with those fed the low-ME diet. Likewise, broilers fed the normal-ME diet had higher abdominal fat^{21,24} and liver weights²⁴ than those fed the low-ME diet. Feeding a diet with normal ME may promote greater triglyceride accumulation in the liver and body, resulting in increased RW of the liver and abdominal fat compared with feeding a low-ME diet³⁸. In contrast, some previous studies have shown that the RW of abdominal fat^{6,22}, liver^{6,10,19,22} and intestine⁶ in broilers are not affected by dietary ME levels. The variations in the RW of abdominal fat and liver may arise from differences in the ME levels of the broiler diets. Moreover, dietary energy levels significantly influence the function and development of the pancreas in broilers, although their effects on relative pancreas weight are often nuanced. When the ME level of the diet was high, broilers reduced their FI to meet their energy requirements. The decreased FI may reduce the overall demand for pancreatic

enzyme secretion, which may consequently result in a reduction in the relative weight of the pancreas³⁹. Moreover, high-ME diets in some studies may have decreased the mRNA expression of pancreatic lipase and colipase, which may be accompanied by structural changes and a decrease in relative pancreas weight (weight relative to total body weight) compared with a low-ME diet⁴⁰. In addition, the three-way interaction of LS×MEL×LL on the RW of the pancreas in broilers was significant (Table 5). LL supplementation in the normal-ME diet containing SO resulted in a reduction in the RW of the pancreas in broilers ($p<0.01$). Supplemental emulsifiers in high-ME diets increase the dispersion of dietary fat droplets into smaller particles (micelles), thereby increasing the surface area available for pancreatic lipase activity and facilitating the digestion and absorption of dietary lipids. Consequently, as dietary lipids are digested more efficiently, the demand for intensive lipase secretion by the pancreas decreases, resulting in a reduced physiological workload and relative pancreas weight³⁵.

In the present study, dietary LL supplementation decreased the RW of abdominal fat ($p<0.05$), liver ($p<0.05$), and small intestine ($p<0.05$) in broilers compared with those fed diets without LL. Our findings are in agreement with those of Ge *et al.*²³ and Cai *et al.*²², who reported that emulsifier supplementation significantly decreased the abdominal fat and liver percentages of broilers at 42 days of age. The reduction in the RW of abdominal fat and liver may also suggest that dietary emulsifier supplementation could promote the utilization of nutrients for muscle growth rather than body and hepatic fat deposition by improving nutrient digestibility^{22,23}.

Serum biochemical parameters: The effects of the experimental treatments on the serum biochemical parameters of broilers at 42 days of age are presented in Table 6.

Neither the main effects of LS, ME level and LL, nor their two-way interactions, LS×MEL, MEL×LL and LS×LL, or the three-way interaction, LS×MEL×LL, significantly affected serum ALT, AST, triglyceride, total cholesterol, or VLDL cholesterol levels in broilers (Table 6). Moreover, dietary ME level and supplemental LL level, as well as their two-way interactions, LS×MEL and MEL×LL and the three-way interaction, LS×MEL×LL, did not significantly affect serum HDL cholesterol levels in broilers (Table 6).

However, diets containing SO increased serum HDL cholesterol levels in broilers compared with those fed diets containing TF ($p<0.001$). This result concurs with the finding reported by Majdollahseini *et al.*²⁴, who found that broilers fed

SO-containing diets had significantly higher serum HDL levels than those fed PF-containing diets. PUFAs, particularly omega-3 fatty acids in vegetable oils, may increase the antioxidant potential of HDL by enhancing the activity of protective HDL enzymes, such as glutathione peroxidase 3, thereby increasing serum HDL cholesterol levels⁴¹. The result obtained in the present study is not consistent with those of Fascina *et al.*³¹ and Neto *et al.*³⁶, who found no differences in serum HDL cholesterol levels between broilers fed diets containing vegetable oil or animal fat. These results are consistent with those of Neto *et al.*³⁶, who reported that serum total cholesterol and triglyceride levels in broilers were not affected by dietary fat sources. In contrast, Fascina *et al.*³¹ reported that feeding a diet containing SO significantly reduced serum triglyceride, total cholesterol and VLDL cholesterol levels in broilers compared with those fed a diet containing beef tallow.

Furthermore, the two-way interaction of LS×LL influenced serum HDL cholesterol levels in broilers ($p<0.05$) (Table 6). Regardless of ME level, serum HDL cholesterol levels in broilers increased ($p<0.05$) following LL supplementation in diets containing SO and decreased ($p<0.05$) following LL supplementation in diets containing TF compared with the corresponding diets without supplemental LL. Unsaturated fats increase lipoprotein lipase (LPL) activity more effectively than saturated fats. LPL is a crucial enzyme located on the capillary endothelium of adipose and muscle tissues. Increased LPL activity promotes the hydrolysis of triglyceride-rich lipoproteins, including VLDL and chylomicrons, into free fatty acids (FAs). Nutritional emulsifiers have been shown to increase LPL activity by increasing the surface area available for lipid digestion and turnover. In the present study, the combined supplementation of vegetable oil rich in UFAs and lysolecithin (emulsifier) increased serum HDL cholesterol levels in broilers, potentially due to enhanced LPL activity and increased lipid surface area, which may promote triglyceride utilization and consequently increase serum HDL cholesterol levels⁴². Our results regarding serum HDL cholesterol levels differ from those reported by Neto *et al.*³⁶ and Majdollahseini *et al.*²⁴, who found no significant differences in serum HDL cholesterol and triglyceride levels between broilers fed diets containing SO or PF with supplemental emulsifiers. The differences in serum HDL cholesterol responses may be attributed to differences in the levels and types of emulsifiers incorporated into broiler diets in previous studies.

Nutrient digestibility: The effects of the experimental treatments on the apparent digestibility (AD) of dry matter (DM), crude protein (CP) and crude fat (CF) in broilers at 42

Table 5: The effects of the experimental treatments on the carcass yields and the relative weight (RW) of some internal organs of broilers at 42 days¹ (%)

Lipid source	ME level	Lysolecithin		Hot		Cold		Abdominal		Small		Pancreas	
		(LL) Level	carcass yields	carcass yields	fat	Heart	Liver	Gizzard	Spleen	intestine	Bile duct	Proventriculus	Pancreas
Soybean oil (SO)	Normal	0 kg/t	76.08	72.27	1.55	0.46	1.78	1.75	0.09	2.88	0.09	0.34	0.17 ^d
		1 kg/t	75.85	72.05	1.54	0.40	1.77	1.80	0.09	2.94	0.11	0.35	0.16 ^e
Soybean oil (SO)	Low	0 kg/t	74.60	70.87	1.65	0.47	1.76	1.71	0.11	3.04	0.12	0.35	0.18 ^c
		1 kg/t	75.68	71.89	1.49	0.42	1.70	1.74	0.11	2.82	0.10	0.33	0.20 ^a
Turkey fat (TF)	Normal	0 kg/t	75.33	71.56	1.86	0.43	1.79	1.81	0.10	2.86	0.10	0.37	0.15 ^f
		1 kg/t	75.81	72.01	1.89	0.43	1.74	1.71	0.09	2.66	0.09	0.32	0.15 ^f
Turkey fat (TF)	Low	0 kg/t	75.25	71.49	1.72	0.45	1.66	1.70	0.10	3.06	0.09	0.37	0.17 ^d
		1 kg/t	75.11	71.35	1.50	0.44	1.61	1.80	0.09	3.03	0.09	0.33	0.19 ^b
SEM			0.136	0.129	0.040	0.008	0.023	0.030	0.003	0.050	0.004	0.005	0.004
Lipid source (LS)													
SO			75.55	71.77	1.56 ^b	0.44	1.75 ^a	1.75	0.10	2.92	0.11	0.34	0.18
TF			75.38	71.61	1.74 ^a	0.44	1.70 ^b	1.76	0.09	2.90	0.09	0.35	0.17
SEM			0.186	0.176	0.053	0.011	0.032	0.041	0.004	0.07	0.005	0.007	0.006
ME level (MEL)													
Normal			75.77	71.98	1.71 ^a	0.43	1.77 ^a	1.77	0.09	2.84 ^b	0.10	0.35	0.16 ^b
Low			75.16	71.40	1.59 ^b	0.44	1.68 ^b	1.74	0.10	2.99 ^a	0.10	0.35	0.19 ^a
SEM			0.186	0.176	0.053	0.011	0.032	0.041	0.004	0.07	0.005	0.007	0.006
Lysolecithin (LL) level (kg/t)													
0 (LL0)			75.32	71.55	1.70 ^a	0.45	1.75 ^b	1.74	0.10	2.96 ^a	0.10	0.36	0.17
1 (LL1)			75.61	71.83	1.60 ^b	0.42	1.71 ^a	1.76	0.09	2.86 ^b	0.10	0.33	0.17
SEM			0.186	0.176	0.053	0.011	0.032	0.041	0.004	0.07	0.005	0.007	0.006
p-value													
LS			0.510	0.510	0.015	0.901	0.003	0.130	0.413	0.867	0.066	0.519	0.120
MEL			0.250	0.250	0.032	0.320	0.006	0.985	0.230	0.013	0.682	0.991	0.002
LL			0.268	0.268	0.022	0.080	0.031	0.927	0.140	0.033	0.603	0.190	0.262
LS × MEL interaction			0.408	0.408	0.052	0.862	0.351	0.460	0.185	0.197	0.654	0.961	0.992
MEL × LL interaction			0.512	0.512	0.185	0.804	0.762	0.108	0.964	0.817	0.293	0.495	0.241
LS × LL interaction			0.622	0.622	0.918	0.147	0.923	0.927	0.397	0.868	0.979	0.053	0.755
LS × MEL × LL interaction			0.071	0.071	0.723	0.737	0.797	0.060	0.871	0.264	0.080	0.249	0.005

^{a-f}Values in the same column not sharing a common superscript differ significantly (*p<0.05, **p<0.01), SEM: Standard Error of the Mean and ¹Data are means of eight broilers per treatment group

Table 6: The effects of the experimental treatments on serum biochemical parameters of broilers at 42 days

						VLDL	Toplam	
						Cholesterol	Cholesterol	Triglyceride
Lipid source	ME level	Lysolecithin (LL) Level	ALT (IU/L)	AST (IU/L)	HDL (mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
Soybean oil (SO)	Normal	0 kg/t	2.567	284.714	91.000 ^c	6.625	128.017	31.800
		1 kg/t	2.933	298.843	97.338 ^a	6.125	125.520	31.013
Soybean oil (SO)	Low	0 kg/t	3.125	272.050	87.313 ^e	7.375	127.000	34.986
		1 kg/t	2.586	299.175	93.438 ^b	6.875	134.800	34.525
Turkey fat (TF)	Normal	0 kg/t	1.767	283.750	90.413 ^{cd}	6.125	133.813	32.029
		1 kg/t	2.900	277.871	79.000 ^g	7.000	120.938	33.975
Turkey fat (TF)	Low	0 kg/t	2.557	293.686	80.838 ^f	6.750	121.133	34.980
		1 kg/t	2.500	281.463	79.200 ^g	6.500	120.888	33.971
SEM			0.201	8.714	1.829	0.191	2.303	0.870
Lipid source (LS)								
SO			2.803	288.700	92.272 ^a	6.750	128.834	33.081
TF			2.431	284.192	82.112 ^b	6.594	124.193	33.739
SEM			0.203	8.728	1.832	0.197	2.315	0.877
ME level (MEL)								
Normal			2.542	286.295	89.437	6.469	127.070	32.204
Low			2.692	286.593	84.947	6.875	125.955	34.616
SEM			0.203	8.728	1.832	0.197	2.315	0.877
Lysolecithin (LL) level (kg/t)								
0 (LL0)			2.504	283.550	87.391	6.719	127.491	33.449
1 (LL1)			2.730	289.338	86.94	6.625	125.536	33.371
SEM			0.203	8.728	1.832	0.197	2.315	0.877
p-value								
LS			0.203	0.717	0.000	0.578	0.164	0.599
MEL			0.603	0.981	0.089	0.151	0.735	0.058
LL			0.436	0.641	0.879	0.738	0.554	0.950
LS×MEL Interaction			0.876	0.603	0.789	0.223	0.116	0.454
MEL×LL Interaction			0.075	0.893	0.412	0.318	0.087	0.599
LS×LL Interaction			0.283	0.235	0.013	0.151	0.167	0.662
LS×MEL×LL Interaction			0.806	0.697	0.389	0.318	0.860	0.512

^{a-g}Values in the same column not sharing a common superscript differ significantly (*p<0.05, ***p<0.001), SEM: Standard Error of the Mean, ¹Data are means of eight broilers per treatment group

days of age are presented in Table 7. Neither the main effects of LS, ME level and LL, nor their two-way interactions, LS×MEL and LS×LL, significantly affected DM digestibility in broilers at 42 days of age (Table 7).

These observations are consistent with the findings of Zhang *et al.*¹⁵, who reported that the AD of DM in broilers was not affected by dietary fat sources (FS) (soybean oil or poultry fat), dietary emulsifier (lysophosphatidylcholine [LPC]) supplementation, or the interaction between FS×LPC. This result is also in agreement with a recent study conducted by Cai *et al.*²², who found that dietary ME levels and the interaction between ME×emulsifier did not influence the AD of DM in broilers. Contrary to our findings, Kamran *et al.*³⁷ reported that broilers fed a diet containing SO had higher AD of DM than those fed a diet containing PF. The same researchers also found that supplementation with an emulsifier in a diet containing SO significantly increased the AD of DM in broilers. The discrepancy in DM digestibility results may be attributed to differences in the level and type of emulsifier supplemented to the diet and the levels of LS included in the diet in previous studies.

However, AD of DM in broilers at 42 days of age was influenced (p<0.05) by the two-way interaction of MEL×LL. Supplementation with LL in a normal-ME diet significantly increased DM digestibility in broilers compared with that in broilers fed a normal-ME diet without LL, whereas supplementation with LL in a low-ME diet significantly decreased DM digestibility compared with that in broilers fed a low-ME diet without LL (Tables 7 and 8).

In contrast to our results, Oketch *et al.*¹⁰, Ko *et al.*²¹, Cai *et al.*²², Majdollahseini *et al.*²⁴ and Ahmadi-Sefat *et al.*³⁴, reported that the two-way interaction between MEL and emulsifier level did not affect DM digestibility in broilers. In the present study, the increase in AD of DM observed following LL supplementation in a normal-ME diet may be attributed to enhanced lipid digestion and absorption efficiency. Emulsifiers reduce the size of fat globules, thereby increasing the surface area available for lipase activity and promoting the formation of mixed micelles. This process facilitates the transport of fatty acids and monoglycerides to enterocytes, improving lipid absorption and optimizing energy utilization in normal-ME diets. An increase in lipid digestion and ME availability can

Table 7: The effects of the experimental treatments on the apparent digestibility (AD) of dry matter (DM), crude protein (CP) and crude fat (CF) of broilers at 42 days

Lipid source	ME level	Lysolecithin (LL) level	Apparent digestibility (AD) (%)		
			DM	CP	CF
Soybean oil (SO)	Normal	0 kg/t	60.178 ^c	70.428	74.243
		1 kg/t	52.111 ^g	74.665	77.440
Soybean oil (SO)	Low	0 kg/t	64.884 ^b	67.218	72.678
		1 kg/t	57.977 ^e	73.478	76.345
Turkey fat (TF)	Normal	0 kg/t	53.939 ^f	68.183	69.668
		1 kg/t	67.603 ^a	59.370	75.445
Turkey fat (TF)	Low	0 kg/t	64.504 ^b	61.553	65.020
		1 kg/t	59.157 ^d	59.283	72.005
SEM			1.136	1.336	1.029
Lipid source (LS)					
SO			58.458	71.447 ^a	75.117 ^a
TF			61.631	62.097 ^b	70.535 ^b
SEM			1.549	1.376	1.225
ME level (MEL)					
Normal			58.788	68.162	74.199 ^a
Low			61.301	65.383	71.512 ^b
SEM			1.549	1.376	1.225
Lysolecithin (LL) Level (kg/t)					
0 (LL0)			60.876	66.846	70.402 ^b
1 (LL1)			59.212	66.699	75.309 ^a
SEM			1.549	1.376	1.225
p-value					
LS			0.161	0.000	0.014
MEL			0.263	0.166	0.013
LL			0.455	0.941	0.010
LS×MEL Interaction			0.345	0.768	0.442
MEL×LL Interaction			0.014	0.282	0.811
LS×LL Interaction			0.053	0.011	0.040
LS×MEL×LL Interaction			0.030	0.567	0.916

^{a-g} Values in the same column not sharing a common superscript differ significantly (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), SEM: Standard Error of the Mean, ¹ Data are means of eight broilers per treatment group

reduce fecal nutrient losses and improve the absorption of fat-soluble vitamins, thereby contributing to improvements in DM digestibility⁴³. Moreover, the three-way interaction of LS×MEL×LL affected DM digestibility in broilers at 42 days of age ($p < 0.05$) (Table 7). Supplementation with LL to a normal-ME diet containing SO resulted in the lowest DM digestibility in broilers, whereas the highest DM digestibility was observed in broilers supplemented with LL in a normal-ME diet containing TF ($p < 0.05$).

As indicated in Table 7, neither the main effects of ME level and LL level, nor their two-way interactions, LS×MEL and MEL×LL, or the three-way interaction, LS×MEL×LL, affected CP digestibility in broilers at 42 days of age. However, feeding a diet containing SO enhanced CP digestibility in broilers at 42 days of age ($p < 0.001$) compared with that in broilers fed a diet containing TF. This finding is in agreement with the results reported by Jansen *et al.*¹⁸, who observed that broilers fed a diet containing SO had higher nitrogen digestibility and retention than those fed a diet containing pig lard. Vegetable oils (SO) rich in UFAs may lead to a lower pH in the small intestinal contents and decrease the levels of pathogenic

bacteria, resulting in less intestinal tissue damage compared with animal fats (TF) rich in SFAs²⁴. As a result, feeding a diet containing SO rich in UFAs may enhance CP digestibility by improving villus height (VH), the villus height (VH):crypt depth (CD) ratio, and villus surface area in the jejunum²⁴, or villus height and the villus height:crypt depth ratio in the ileum of broilers compared with TF rich in SFAs². Contrary to our findings, Zhang *et al.*¹⁵, Majdolhosseini *et al.*²⁴ and Kamran *et al.*³⁷ reported that CP digestibility in broilers was not affected by fat source. Furthermore, Neto *et al.*³⁶ reported that broilers fed a diet containing SO had significantly lower CP digestibility than those fed a diet containing PF. The discrepancy in CP digestibility results may arise from differences in the inclusion levels and fatty acid profiles of LSs in the diets.

The CP digestibility of broilers at 42 days of age was influenced ($p < 0.05$) by the two-way interaction of LS×LL (Table 7 and 9). CP digestibility in broilers was enhanced ($p < 0.05$) by LL supplementation in diets containing SO, but decreased ($p < 0.05$) by LL supplementation in diets containing TF compared with the corresponding diets without

supplemental LL. The greater digestion and absorption of vegetable oils rich in UFAs following lysolecithin supplementation might decrease the proliferation of pathogenic bacteria in the small intestine, leading to reduced villus surface damage and increases in VH and the VH:CD ratio in the ileum, and consequently improving CP digestibility in broilers². However, our results did not concur with the findings of Majdolhosseini *et al.*²⁴, who reported that the interaction between dietary emulsifier supplementation and LS was not significant for CP digestibility in broilers.

Diet containing SO increased CF digestibility in broilers compared with that of broilers fed a diet containing TF ($p < 0.05$) (Table 7). Our finding is supported by the results of Majdolhosseini *et al.*²⁴, Kamran *et al.*³⁷ and Ye *et al.*⁴⁴, who reported that SO-containing diets increased CF digestibility in broilers compared with PF-containing diets. The digestibility of CF from dietary LSs is dependent on the lipid source and differences in the degree of fatty acid saturation. Vegetable oils rich in UFAs, such as SO, are more readily incorporated into micelles than animal fats rich in SFAs, such as PF and TF, because of their greater potential for emulsification in the presence of adequate bile salts and lipase activity^{15,45}. Furthermore, differences in CF digestibility may be attributed to variations in the fatty acid profiles of dietary lipid sources. Vegetable oils (SO) rich in UFAs may reduce the pH of the small intestinal contents and decrease the abundance of pathogenic bacteria, resulting in less intestinal tissue damage compared with animal fats, such as TF, which are rich in SFAs. Consequently, diets containing SO rich in UFAs may enhance CF digestibility by improving intestinal morphology, as evidenced by an increased villus height-to-crypt depth (VH:CD) ratio and greater villus surface area in the jejunum of broilers compared with diets containing TF rich in SFAs²⁴.

As indicated in Table 7, the normal-ME diet increased CF digestibility in broilers compared with that in broilers fed the low-ME diet ($p < 0.05$). Our finding concurs with the results of Hu *et al.*³⁵, Ko *et al.*²¹ and Jalal *et al.*²⁰, who reported that broilers fed normal-ME diets had significantly higher CF digestibility than those fed low-ME diets. In the current study, broilers fed reduced-ME diets increased their FI to compensate for the reduced ME content and maintain growth performance, which may have resulted in decreased CF digestibility. Increased FI resulting from reduced dietary ME may enhance the passage rate of feed through the gastrointestinal tract, leaving less time for the enzymatic degradation of fats. It may also increase the workload of the gastrointestinal tract, leading to less efficient emulsification and reduced lipase activity relative to the cumulative amount of feed consumed²¹. Our result is not in agreement with

Table 8: The effects of two-way interactions of MEL×LL on dry matter digestibility in broilers (%)

ME level (MEL)	Lysolecithin level (LL)	Dry matter digestibility
Normal	0 kg/t	^B 57.059 ^B
	1 kg/t	^B 59.857 ^A
Low	0 kg/t	^A 62.021 ^A
	1 kg/t	^A 61.241 ^B
p-value		0.014

^{a-b}The lowercase letters on the right show two-way interactions of MEL×LL

^{A-B}The capital letters on the left show two-way interactions of LL×MEL

Table 9: The effects of two-way interactions of LS×LL on CP digestibility in broilers (%)

Lipid source (LS)	Lysolecithin level (LL)	CP digestibility
SO	0 kg/t	^A 68.823 ^B
	1 kg/t	^A 74.072 ^A
TF	0 kg/t	^B 64.868 ^A
	1 kg/t	^B 59.327 ^B
p-value		0.011

^{a-b}The lowercase letters on the right show two-way interactions of LS×LL

^{A-B}The capital letters on the left show two-way interactions of LL×LS

previous studies reported by Majdolhosseini *et al.*²⁴, Kamel *et al.*⁶, Ahmadi-Sefat *et al.*³⁴, Ahmad *et al.*⁴⁶ and Cai *et al.*²², who showed that fat digestibility in broilers was not affected by the dietary ME level. The discrepancy in CF digestibility results may be due to differences in the ME levels of the diets.

LL supplementation increased CF digestibility in broilers compared with that in broilers fed diets without supplemental LL ($p < 0.05$) (Table 7). This result concurs with the findings reported by Oketch *et al.*¹⁰ and Jalal *et al.*²⁰, Majdolhosseini *et al.*²⁴, and Ahmadi-Sefat *et al.*³⁴, who found that emulsifier supplementation significantly enhanced fat digestibility in broilers compared with diets without supplemental emulsifiers. Emulsifiers such as lysolecithin reduce the surface tension between lipid droplets and water, enhancing micelle formation, which is essential for pancreatic lipase activity in the small intestinal lumen and facilitates the absorption of fat or oil in the small intestine, thereby increasing CF digestibility²¹. Conflicting results regarding the effect of emulsifier supplementation on fat digestibility have been reported by Ye *et al.*⁴⁴, Ko *et al.*²¹, Oliveira *et al.*⁴⁷ and Cai *et al.*²², who found that dietary emulsifier supplementation had no significant effect on fat digestibility. The variation in these results may be attributed to differences in the type and dietary inclusion level of the emulsifier and experimental conditions, such as broiler strain, among studies.

The CF digestibility of broilers at 42 days of age was influenced ($p < 0.05$) by the two-way interaction of LS×LL (Table 7 and 10).

Supplementation of LL to the diet containing TF increased ($p < 0.05$) CF digestibility, whereas LL supplementation to the diet containing SO did not affect CF digestibility in broilers. In

Table 10: The effects of two-way interactions of LS×LL on CF digestibility in broilers (%)

Lipid source (LS)	Lysolecithin level (LL)	CF digestibility
SO	0 kg/t	^A 73.641 ^a
	1 kg/t	^A 76.893 ^a
TF	0 kg/t	^B 67.344 ^b
	1 kg/t	^A 73.725 ^a
p-value		0.040

^{a-b}The lowercase letters on the right show two-way interactions of LS×LL

^{A-B}The capital letters on the left show two-way interactions of LL×LS

agreement with our finding, supplementation of emulsifiers to PF-containing diets has been shown to significantly enhance CF digestibility in broilers²⁴. In the present study, the increase in CF digestibility following emulsifier supplementation in the TF-containing diet may be related to the emulsification properties of the emulsifier. TF is reported to be less readily emulsified by native bile salts in the digestive tract because of its physicochemical properties. Emulsifier supplementation, particularly in TF-containing diets, may enhance the emulsification process by improving the stabilization and clearance of lipid droplet surfaces through bile salts. This, in turn, facilitates lipase attachment at the lipid-water interface, thereby improving CF digestibility in broilers fed TF-containing diets²⁴. The use of a highly digestible lipid source, such as soybean oil, in the diet may have left limited scope for additional emulsifier activity, thereby limiting its effect on the digestibility of vegetable oil such as SO⁴⁸.

CONCLUSION

The results of the present study demonstrate that the use of TF and the reduction of dietary ME by 100 kcal/kg had adverse effects on the growth performance of broilers. However, dietary supplementation with the exogenous emulsifier lysolecithin (LL) had positive effects on the growth performance of broilers throughout the overall experimental period. Diets containing SO increased serum HDL cholesterol levels and the digestibility of crude protein (CP) and crude fat (CF) in broilers compared with those fed diets containing TF. Serum HDL cholesterol levels and CP digestibility were increased by LL supplementation in diets containing SO, but decreased by LL supplementation in diets containing TF compared with the corresponding diets without supplemental LL. In addition, LL supplementation in diets containing TF increased CF digestibility, whereas LL supplementation in diets containing SO did not affect CF digestibility in broilers. Consequently, further studies investigating the effects of LS, ME level, and supplemental LL level on the histological parameters of the small intestine are warranted to clarify the mechanisms underlying their effects on the evaluated variables.

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