Effects of dietary fat source and supplemental lysophosphatidylcholine on performance, immune responses, and ileal nutrient digestibility in broilers fed corn/soybean meal- or corn/wheat/soybean meal-based diets

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ABSTRACT Two separate experiments were conducted to investigate the effect of different fat sources and a supplemental exogenous emulsifier (lysophosphatidylcholine, LPC) on growth performance, antibody production titers, and ileal nutrient digestibility in broiler chicks fed with different basal diets. A total of 288 one-day-old Ross 308 chicks were used for each trial (6 dietary treatments based on 3 × 2 factorial arrangements of treatments in both trials) with 4 replicates of 12 birds each. Dietary treatments consisted of 3 different fat sources (soy oil, SO; soy free fatty acids, SFFA; and palm fat powder, PFP) and 2 LPC levels (0 and 0.1% of diet), which were evaluated with 2 different basal diets (corn/soybean meal-based diets in Exp. 1, or corn/wheat/soybean meal-based diets in Exp. 2). In Exp. 1, average daily feed intake (ADFI) was increased ($P < 0.01$) in birds fed PFP diets compared with those fed SO or SFFA diets. Although supplemental LPC decreased ($P < 0.01$) ADFI, the birds fed SFFA diets had the greater ADFI at the presence of LPC (fat source × LPC, $P < 0.01$). Dietary supplementation of LPC caused a 4.6% improvement ($P < 0.001$) in average daily weight gain (ADWG) and consequently improved ($P < 0.01$) feed conversion ratio (FCR). Supplemental LPC was more effective in increasing ADWG in SFFA-containing diets, resulted in a significant ($P < 0.01$) dietary fat source × LPC interaction. Dietary inclusion of LPC increased ($P < 0.01$) bursa weight and improved ($P < 0.05$) antibody production titers against sheep red blood cells and Newcastle disease virus during primary responses. Ileal digestibility of ether extract (EE) was improved ($P < 0.05$) in birds fed diets containing SO as compared with those fed PFP diets; dietary LPC supplementation, however, had no marked effect on ileal nutrient digestibility. In Exp. 2, ADWG was greater ($P < 0.05$) in birds fed SO-containing diets compared with PFP-supplemented broiler chicks. Furthermore, dietary supplementation with LPC improved ($P < 0.05$) FCR value by 2.1%. Relative thymus weight was greater ($P < 0.05$) in birds fed LPC-supplemented diets than those fed unsupplemented diets. Supplemental LPC increased ($P < 0.05$) Gumboro antibody titer, and the lowest antibody response was allotted to the birds fed PFP diets. The greatest ($P < 0.05$) EE digestibility was assigned to the birds fed SO and SFFA diets. The present findings showed that birds fed SFFA-containing diets had similar performance as SO birds, and supplemental LPC improved overall performance especially in SFFA-fed birds.

Key words: broiler chick, dietary fat source, lysolecithin emulsifier, antibody titer, ileal nutrient digestibility

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INTRODUCTION

Since the 1950s, many studies have been conducted to evaluate the nutritional value of different fat sources for poultry. Sunde (1956) showed that the addition of fat into poultry diets improved growth performance and feed efficiency. Fats are the best and most condensed energy sources, and their inclusion in poultry diets is the simplest method for increasing the energy density (Leeson and Summers, 2005). Vegetable oils, including soybean oil (**SO**), canola oil, corn oil, and sunflower oil, are used as the most common fat sources in poultry diets. It has been reported that dietary replacement of tallow by vegetable oils rich in polyunsaturated fatty acids (such as SO or linseed oil) improved performance and decreased abdominal fat deposition in broiler chicks (Crespo and Esteve-Garcia, 2002; Ferrini et al., 2008; Wongsuthavas et al., 2008). Compared to these oil sources, which are commonly used for human consumption, alternative fat sources such as recycled oils, soy free fatty acids (**SFFA**), yellow grease, and palm fat powder (**PFP**) are relatively inexpensive (Irandoust et al., 2014; Jahanian and Rasouli, 2014b). Therefore, using these fat supplements as energy sources in poultry diets could reduce the feed

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cost, consequently improving the profitability of poultry farms. In this regard, Jahanian and Rasouli (2014b) reported that SO can be replaced by PFP by 75% without any detrimental effect on performance and egg quality indices in laying hens.

It has been well known that fat digestion is facilitated by the combined action of bile acids, lipase, and a protein called co-lipase (Leeson and Summers, 2001). It was demonstrated that the physiological functions necessary for efficient fat digestion are not properly developed in young chickens and continue to develop for several weeks after hatching (Jin et al., 1998). Noy and Sklan (1995) reported that lipase secretion was low at hatching and increased 20-fold between 4 to 21 d of age in broiler chickens. Because the younger birds have insufficient secretions of endogenous bile salts and lipase, dietary supplementation of exogenous emulsifiers may improve fat utilization within the gastrointestinal tract. Abbas et al. (2016) observed that dietary supplementation of lecithin, as an emulsifier, increased overall weight and fat digestibility in broiler chicks. Furthermore, Maisonnier et al. (2003) reported that dietary inclusion of bile salts increased lipid digestibility, with more impact in conventional birds (the greater intestinal microflora) and in birds fed guar gum diets.

On the other hand, one of the main issues in poultry production is the cost of feed ingredients such as corn and soybean meal. Corn price has increased during recent years (Donohue and Cunningham, 2009), mainly because of its utilization in other industries such as ethanol production (Aho, 2007). An alternative for corn in poultry diets is wheat; however, the higher levels of water-soluble non-starch polysaccharides (**NSP**), including arabinoxylans, are known to exert adverse effects on performance and nutrient digestibility in broilers fed wheat-containing diets (Bedford and Classen, 1992). Such negative effects are thought to be caused by an increased digesta viscosity (Steenfeldt et al., 1998a,b; Jahanian and Rasouli, 2014a). Several studies have shown that, among the nutrients, fat digestion suffers the most pronounced impairment due to high digesta viscosity (Ward and Marquardt, 1983; Steenfeldt et al., 1998a; Maisonnier et al., 2003). Furthermore, an increase in intestinal viscosity is more detrimental for digestion and absorption of those dietary fat sources, which contain higher proportions of saturated fatty acids (**SFA**).

Efforts to ameliorate the negative impacts of the anti-nutritional components of wheat on fat digestion by supplemental emulsifiers can be useful. On the other hand, using the cheaper fat sources in poultry diets can increase economic efficiency in poultry farms through reducing feed costs. Therefore, the objectives of these studies were to investigate the effects of dietary supplementation of an exogenous emulsifier (lysophosphatidylcholine, **LPC**) on performance, lymphoid organs weight, immune responses, and ileal nutrient digestibility in broiler chicks fed different basal diets and various supplemental fat sources.

MATERIALS AND METHODS

Birds, Experimental Procedure, and Dietary Treatments

The present studies were conducted in the Poultry Research Station of Isfahan University of Technology (Isfahan, Iran) and all experimental procedures were approved by the Isfahan University of Technology Animal Care and Use Committee. A total of 288 one-day-old Ross 308 broiler chicks (mixed sex) were used in each trial, and chicks were randomly distributed among 6 dietary treatments with 4 replicates of 12 chicks each. All experimental pens had equal initial weights and weight distribution. Pens were rechecked at 2 wk of age to ensure that there were similar numbers of males and females among different experimental groups. Chicks were housed in floor pens (1 × 1 m) covered with carton rolls in an environmentally controlled room. Each pen was equipped with a pan feeder and a manual bell-shaped drinker. The experimental periods lasted 42 days. In both experiments, the birds were given ad libitum access to water and to the experimental diets. Light was on continuously during the first wk; thereafter, a 23L:1D lighting schedule was used for the duration of the experiment. Temperature was set at 34°C during the first week of age; thereafter, it was reduced by 3°C/wk until the chicks were 5 wk old.

Dietary treatments included 3 different fat sources (SO, SFFA, and PFP) and 2 levels (0 and 0.1% of diet) of LPC (Easy Bio Co., Gangnam-gu, Seoul, Korea), which were fed to the birds according to the 3 × 2 factorial arrangements of treatments. This treatment pattern was used with 2 different basal diets during 2 separate trials: a corn/soybean meal-based diet in Exp. 1 and a corn/wheat/soybean meal-based diet in Exp. 2. Experimental diets (Table 1) were formulated to meet the nutrient requirements of birds as provided by the *Ross* Broiler Management Manual (2009) during starter (1 to 14 d of age), grower (15 to 28 d of age), and finisher (29 to 42 d of age) periods. All of the diets were similar in nutrient composition during each growth period.

Before formulating the diets, feed ingredients (i.e., corn, wheat, soybean meal, and corn gluten meal) were analyzed for dry matter (Method 934.01), crude protein (**CP**; Method 976.06), ether extract (**EE**; 954.02), and total ash (Method 942.05) according to the standard procedures of the Association of Official Analytical Chemists (2002). Supplemental fat sources were analyzed for their fatty acid composition by gas chromatography (**GC**) according to the method developed by López-Ferrer et al. (1999) and results are shown in Table 2. In addition, the peroxide value of dietary fat sources was measured by the standard method

Table 1. Ingredients and nutrient composition of experimental diets during different growth periods.

Items (% unless stated otherwise)	Experiment 1			Experiment 2		
	Starter (1 to 14 d)	Grower (15 to 28 d)	Finisher (29 to 42 d)	Starter (1 to 14 d)	Grower (15 to 28 d)	Finisher (29 to 42 d)
Ingredients						
Corn, yellow	52.42	56.82	62.39	39.13	34.61	35.64
Soybean meal	39.16	36.63	31.13	36.82	32.81	26.64
Corn gluten meal	2.00	–	–	2.00	–	–
Wheat, red hard	–	–	–	15.00	25.00	30.00
Soy oil[1]	1.91	2.27	2.35	2.44	3.19	3.47
Monocalcium phosphate	1.43	1.33	1.29	1.42	1.31	1.27
Limestone	1.74	1.67	1.59	1.75	1.68	1.61
Common salt	0.24	0.24	0.24	0.24	0.23	0.23
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.18	0.17	0.16	0.18	0.17	0.16
L-Lysine-HCl	0.05	0.02	–	0.12	0.13	0.10
L-Threonine	0.02	–	–	0.05	0.02	0.03
Zeolite[4]	0.20	0.20	0.20	0.20	0.20	0.20
Nutrient composition[5]						
ME_n (kcal/kg)	2,900	2,950	3,020	2,900	2,950	3,020
Crude protein	22.50	20.50	18.50	22.50	20.50	18.50
Crude protein (analyzed)	22.41	20.63	18.27	22.72	20.54	18.38
Ether extract	4.90	5.40	5.80	5.10	5.80	6.20
Ether extract (analyzed)	5.02	5.23	5.98	5.00	5.73	6.04
Methionine	0.55	0.50	0.46	0.54	0.49	0.46
Methionine + cysteine	0.92	0.84	0.78	0.92	0.84	0.78
Lysine	1.28	1.18	1.03	1.28	1.18	1.00
Threonine	0.88	0.79	0.71	0.88	0.76	0.68
Calcium	1.00	0.95	0.90	1.00	0.95	0.90
Non-phytate P	0.48	0.45	0.43	0.48	0.45	0.43
Sodium	0.15	0.15	0.15	0.15	0.15	0.15

[1]Soy oil was replaced with soy free fatty acids or palm fat powder in other experimental diets. The metabolizable energy values (ME_n) of supplemental fat sources were considered as 9,600, 8,500, and 8,700 kcal/kg for soil oil, soy free fatty acids, and palm fat powder, respectively. Because the supplemental fat sources were different in their ME_n values, the amounts of supplemental fat, corn, and soybean meal were slightly modified, so that the amounts of supplemental fat and soybean meal were increased at the expense of corn in soy free fatty acid- and palm fat powder-supplemented diets.

[2]Mineral premix provided per kilogram of diet: Mn (from $MnSO_4.H_2O$), 80 mg; Zn (from ZnO), 65 mg; Fe (from $FeSO_4.7H_2O$), 50 mg; Cu (from $CuSO_4.5H_2O$), 8 mg; I (from Ca $(IO_3)_2.H_2O$), 1.8 mg; Se, 0.30 mg.

[3]Vitamin premix provided per kilogram of diet: vitamin A (from retinyl acetate), 12,500 IU; cholecalciferol, 3,500 IU; vitamin E (from DL-α-tocopheryl acetate), 35 IU; vitamin B_{12}, 0.06 mg; riboflavin, 5.4 mg; nicotin amide, 50 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidinol), 2.5 mg; folic acid, 0.8 mg; thiamine, 3 mg; pyridoxine, 8 mg; biotin, 0.25 mg; choline (as choline chloride), 560 mg; ethoxyquin, 80 mg.

[4]Emulsifier product (lysophosphatidylcholine, Easy Bio Co., Gangnam-gu, Seoul, Korea) was replaced with equal amount (0.1%) of zeolite.

[5]Calculated nutrient composition (except ether extract content) was similar for all dietary treatments. Dietary concentrations of crude protein and ether extract are based on the analyzed values (before formulation of diets). ME_n = apparent metabolizable energy corrected for zero nitrogen retention.

Table 2. Fatty acid composition and peroxide values of supplemental fat sources.

Fatty acids (mg/g)	Soy oil	Soy free fatty acids	Palm fat powder
C 14:0	1.7	2.5	0.8
C 16:0	105.9	171.6	857.2
C 16:1	nd	nd	nd
C 17:0	0.6	0.8	nd
C 17:1	nd	nd	nd
C 18:0	43.4	89.3	14.2
C 18:1	228.2	265.8	108.5
C 18:2	527.8	403.5	17.9
C 18:3	71.8	52.6	nd
C 20:0	3.6	4.2	nd
C 20:4 (n-6)	1.7	0.9	nd
C 22	3.5	2.8	nd
C 20:5 (n-3)	nd	nd	nd
C 23	2.1	2.3	nd
C 22:6 (n-3)	nd	nd	nd
Free fatty acids (%)	1.62	88.24	3.79
Peroxide value (mEq/kg)	1.03	13.59	0.74

nd: not detected.

of the Association of Official Analytical Chemists (2002). Free fatty acids (**FFA**) content of studied fat sources was determined by a modified FFA method (American Oil Chemists' Society, 2004). Metabolizable energy values of supplemental fat sources were considered as 9,600, 8,500, and 8,700 kcal/kg for SO, SFFA, and PFP, respectively (Jahanian, unpublished data).

In both experiments, performance traits, including average daily weight gain (**ADWG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**), were recorded at biweekly intervals and overall performance parameters were calculated from 1 to 42 d of age. Two randomly selected chickens from each pen replicate (n = 8) were killed by cervical dislocation at 42 d of age; thereafter, the spleen, bursa of Fabricius, and thymus were immediately excised and weighed on a sensitive digital scale. The mean values of 2 birds per pen were

used for analysis of variance as described by Jahanian et al. (2008).

Antibody Responses to Different Antigens

Serum samples were taken after immunization against sheep red blood cells (**SRBC**), Newcastle (**NDV**), infectious bronchitis (**IBV**), and infectious bursal (**IBD**) disease viruses to measure antibody responses. The chickens were vaccinated against viral diseases according to the following program: vaccination against NDV and IBV on d 8, NDV on d 13, and IBD on d 18 (all of them via drinking method). The serum samples were collected on d 7 and 14 after NDV and on d 10 after IBV and IBD inoculations. The hemagglutination inhibition test (Jahanian, 2009) was set up to determine antibody production response against NDV. The anti-IBV and anti-IBD antibody titers were measured according to Nassiri Moghaddam and Jahanian (2009) using the ELISA method. In addition, the birds (wing-banded) were inoculated with 0.5 cc of 5% suspension (in phosphate buffer saline) of SRBC at 24 and 35 d of age; thereafter, blood samples were collected via brachial vein on d 7 post each inoculation and total SRBC antibody titers were determined according to hemagglutination assay (Rasouli and Jahanian, 2015). Antibody titers against SRBC were expressed as the $\log_2$ of the reciprocal of the highest serum dilution giving complete agglutination.

Ileal Digestibility

The acid insoluble ash (**AIA**) marker method of McCarthy et al. (1974) was used to measure ileal nutrient digestibility. Diets were supplemented with 0.5% of an AIA source (Celite®; Celite Corp., Lompoc, CA) and fed to the birds for the last 4 d of each trial. Two birds from each pen replicate were randomly selected and killed by cervical dislocation at 42 d of age. The ileal contents (from 5 cm after Meckel's diverticulum to 4 cm before ileocecal junction) were collected directly into the 80 mL sampling cups. The digesta samples were frozen at −20°C until further analysis could be performed. Freeze-dried digesta and feed samples were ground and analyzed for AIA (McCarthy et al., 1974), CP, EE, and total ash (Association of Official Analytical Chemists, 2002). All samples were analyzed on duplicate.

Statistical Analysis

A two-way ANOVA of the data using the general linear models procedure of SAS statistical software (SAS Institute, 1999) was performed according to a 3 × 2 factorial arrangement of treatments (for each trial), including dietary fat source and LPC level as the main effects and respective interaction. Pen was considered as the experimental unit for all measurements. Differences among treatment means were separated using the Least Significant Difference test at $P < 0.05$ statistical level.

RESULTS

Composition of Supplemental Fat Sources

As shown in Table 2, supplemental fat sources were completely different in their fatty acid composition. While SO and SFFA were enriched by unsaturated fatty acids (**UFA**) like oleic and linoleic acids, the main fatty acid component of PFP was palmitic acid. Overall, both SO and SFFA are categorized as unsaturated fat sources and PFP is a source of SFA. On the other hand, many fatty acids (such as linolenic, arachidic, and arachidonic acids) are not detectable in PFP. In addition to fatty acid composition, experimental fat sources were analyzed for their FFA content and peroxide values. As shown in Table 2, SFFA contained 88.24% FFA, while FFA content of SO and PFP were less than 5%. Furthermore, SFFA had the greatest peroxide value among the studied fat sources.

Performance Parameters

Dietary fat source had no marked effect on ADWG and FCR of broiler chicks in Exp. 1 (Table 3). On the other hand, ADFI ($P < 0.01$) was greater in birds fed diets containing PFP than those fed SO- or SFFA-supplemented diets. The addition of 0.1% LPC to the experimental diets improved ($P < 0.01$) performance parameters. Supplementation of LPC into the diets improved ($P < 0.001$) ADWG by 4.6% on average throughout the trial period. In addition, dietary inclusion of LPC caused an improvement in FCR values by 5.9%. There was a significant ($P < 0.01$) dietary fat source × LPC interaction for ADWG and ADFI, so that dietary LPC supplementation increased ADFI and resultant ADWG in birds fed SFFA-containing diets.

The effects of dietary fat source and LPC supplementation on growth performance of broilers fed wheat-containing diets (Exp. 2) are presented in Table 3. Dietary inclusion of PFP decreased ($P < 0.05$) ADWG compared with SO diets, and the birds fed SFFA-supplemented diets had moderate ADWG, which was not significantly different from 2 other fat sources. Although dietary supplementation of LPC improved ($P < 0.05$) FCR values by 2.1%, it had no significant effect on ADWG. In addition, ADFI was not influenced by dietary treatments.

Lymphoid Organs Weight

The effects of supplemental fat sources and dietary LPC supplementation on relative weights of lymphoid organs are shown in Table 4. Dietary fat sources had no marked effect on the relative weights of lymphoid

Table 3. Effect of lysophosphatidylcholine (LPC) emulsifier on performance parameters (1 to 42 d of age) of broiler chicks fed different fat sources (Experiments 1 and 2).[1]

		Experiment 1			Experiment 2		
Fat sources	LPC[2]	ADWG (g/d per bird)	ADFI (g/d per bird)	FCR (g feed/g gain)	ADWG (g/d per bird)	ADFI (g/d per bird)	FCR (g feed/g gain)
SO	–	54.6^{b,c}	103.0^{b}	1.88	56.5	107.6	1.90
	+	55.9^{b}	96.6^{d}	1.73	56.9	102.0	1.78
SFFA	–	53.7^{c}	99.5^{c}	1.84	55.6	104.1	1.86
	+	59.5^{a}	102.0^{b}	1.74	55.8	102.2	1.85
PFP	–	54.7^{b,c}	105.4^{a}	1.91	53.5	102.3	1.90
	+	55.7^{b}	102.1^{b}	1.83	54.5	102.4	1.88
SE		0.92	1.29	0.07	1.84	2.58	0.05
Fat sources							
SO		55.2	99.8^{b}	1.82	56.7^{a}	104.8	1.84
SFFA		56.0	101.0^{b}	1.81	55.7^{a,b}	103.0	1.86
PFP		55.0	103.5^{a}	1.87	54.0^{b}	102.3	1.89
SE		0.63	0.93	0.04	1.03	1.53	0.03
LPC							
–		54.4^{b}	103.0^{a}	1.88^{a}	55.0	104.1	1.89^{a}
+		56.9^{a}	100.4^{b}	1.77^{b}	55.5	102.2	1.85^{b}
SE		0.54	0.76	0.03	0.75	1.32	0.02
Probability							
Fat sources		0.1026	0.0014	0.1605	0.0348	0.3521	0.1197
LPC		0.0003	0.0068	0.0086	0.4892	0.0751	0.0249
Fat sources × LPC		0.0065	0.0016	0.5643	0.8881	0.2590	0.0943

[1]SO: soy oil; SFFA: soy free fatty acids; PFP: palm fat powder; ADWG: average daily weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio; SE: standard error.

[2]In LPC-supplemented diets (+), emulsifier product (Lipidol, Easy Bio Co., Gangnam-gu, Seoul, Korea) was supplemented to the diet at the level of 0.1%.

[a–d]Means with no common superscripts within the column of each classification (fat sources, LPC, or respective interaction) are significantly ($P < 0.05$) different.

organs in Exp. 1. In contrast, dietary LPC supplementation increased ($P < 0.01$) the relative bursa weight compared with unsupplemented birds. Relative weights of the thymus and spleen, however, were not affected by dietary treatments. In Exp. 2, the birds fed SO or SFFA diets had the lower ($P < 0.01$) thymus weights compared with those on PFP-containing diets. On the other hand, the relative weight of the thymus was increased ($P < 0.05$) as a result of dietary supplementation of LPC. In contrast to the thymus, relative weights of the bursa and spleen were not affected by dietary treatments in Exp. 2. There was a significant ($P < 0.05$) dietary fat source × LPC interaction for spleen weight, so that dietary inclusion of LPC increased spleen weight only when the diets were already supplemented with PFP.

Antibody Titers Against Different Antigens

As shown in Table 5, dietary inclusion of PFP increased ($P < 0.05$) both IBV and SRBC antibody titers compared with SO or SFFA diets in Exp. 1. Furthermore, dietary supplementation of LPC increased ($P < 0.05$) SRBC antibody titer during the primary response and anti-NDV titer at d 7 post vaccine inoculation. In contrast, antibody response to IBD was not affected by dietary treatments. There was no significant interaction between dietary fat source and supplemental LPC for antibody responses in Exp. 1.

The effect of supplemental LPC on antibody titers of birds fed wheat-containing diets (Exp. 2) are shown in Table 6. The lowest ($P < 0.05$) antibody titers against IBD were assigned to the birds fed PFP diets. On the other hand, dietary inclusion of SFFA was associated with the lowest ($P < 0.05$) antibody production titer against NDV. Dietary supplementation of LPC increased ($P < 0.05$) IBD antibody response. There was no marked effect of dietary fat source or LPC on antibody response against IBV.

Ileal Nutrient Digestibility

The effects of dietary fat source and supplemental LPC on ileal nutrient digestibility are shown in Table 7. The greatest ($P < 0.05$) coefficient of EE digestibility in Exp. 1 was allotted to the birds fed SO-supplemented diets, followed by those on SFFA diets. Inclusion of SFFA into the diets decreased ($P < 0.05$) ash digestibility within the ileal digesta. In contrast to dietary fat sources, dietary LPC supplementation was not found to affect ileal nutrient digestibility in Exp. 1. Similarly, ileal EE digestibility was affected ($P < 0.05$) by supplemental fat sources in Exp. 2. The greatest digestibility coefficients were seen in birds fed SO-supplemented diets, followed by those fed SFFA-containing diets. Ileal nutrient digestibility was not affected by supplemental LPC or dietary fat source × LPC interaction.

Table 4. Effect of lysophosphatidylcholine (LPC) emulsifier on lymphoid organs' weights (as the percentage of live body weight) of broiler chickens (at d 42 of age) fed different fat sources (Experiments 1 and 2).[1]

		Experiment 1			Experiment 2		
Fat sources	LPC[2]	Spleen	Thymus	Bursa of Fabricius	Spleen	Thymus	Bursa of Fabricius
SO	–	0.12	0.23	0.06	0.11[b]	0.20[b]	0.09
	+	0.13	0.26	0.08	0.11[b]	0.26[a]	0.09
SFFA	–	0.11	0.22	0.06	0.11[b]	0.20[b]	0.07
	+	0.12	0.24	0.07	0.10[b]	0.22[b]	0.09
PFP	–	0.15	0.24	0.06	0.10[b]	0.26[a]	0.07
	+	0.11	0.25	0.11	0.14[a]	0.25[a]	0.06
SE		0.02	0.03	0.03	0.02	0.02	0.02
Fat sources							
SO		0.12	0.24	0.07	0.11	0.22[b]	0.09
SFFA		0.12	0.23	0.07	0.11	0.21[b]	0.08
PFP		0.13	0.25	0.09	0.12	0.26[a]	0.07
SE		0.01	0.02	0.02	0.01	0.02	0.01
LPC							
–		0.12	0.23	0.06[b]	0.11	0.22[b]	0.08
+		0.12	0.25	0.08[a]	0.12	0.24[a]	0.08
SE		0.01	0.01	0.01	0.01	0.01	0.01
Probability							
Fat sources		0.6620	0.5168	0.1550	0.4814	0.0028	0.2325
LPC		0.7190	0.1216	0.0045	0.2507	0.0215	0.4991
Fat sources × LPC		0.1864	0.7627	0.1705	0.0482	0.0125	0.3628

[1]SO: soy oil; SFFA: soy free fatty acids; PFP: palm fat powder; SE: standard error.
[2]In LPC-supplemented diets (+), emulsifier product (Lipidol, Easy Bio Co., Gangnam-gu, Seoul, Korea) was supplemented to the diet at the level of 0.1%.
[a,b]Means with no common superscripts within the column of each classification (fat sources, LPC, or respective interaction) are significantly ($P < 0.05$) different.

Table 5. Effect of lysophosphatidylcholine (LPC) emulsifier on antibody responses against different antigens in broiler chicks fed different fat sources (Experiment 1).[1]

		SRBC (Log_2)		NDV (Log_2)			
Fat sources	LPC[2]	Primary	Secondary	7 dpi	14 dpi	IBV (Log_{10})	IBD (Log_{10})
SO	–	2.50	3.12	3.93	3.73	3.01	3.66
	+	3.12	3.12	3.93	3.45	3.12	3.91
SFFA	–	2.00	2.37	2.80	3.27	3.37	3.77
	+	3.75	3.16	3.86	3.95	3.45	3.70
PFP	–	4.00	3.87	2.58	2.66	3.67	3.09
	+	6.00	3.87	3.10	3.32	4.07	3.58
SE		1.86	0.80	0.68	0.62	0.52	0.43
Fat sources							
SO		2.81[b]	3.12	3.93[a]	3.59	3.06[b]	3.78
SFFA		2.87[b]	2.71	3.33[a,b]	3.61	3.41[a,b]	3.73
PFP		5.00[a]	3.87	2.84[b]	2.99	3.87[a]	3.34
SE		0.97	0.62	0.43	0.40	0.38	0.29
LPC							
–		2.83[b]	3.12	3.10[b]	3.22	3.35	3.51
+		4.29[a]	3.40	3.63[a]	3.57	3.55	3.73
SE		0.72	0.46	0.31	0.35	0.28	0.25
Probability							
Fat sources		0.0103	0.0829	0.0084	0.1671	0.0340	0.1755
LPC		0.0234	0.4991	0.0500	0.2379	0.4018	0.2916
Fat sources × LPC		0.6056	0.6430	0.2571	0.3206	0.8237	0.5490

[1]SO: soy oil; SFFA: soy free fatty acids; PFP: palm fat powder; SRBC: sheep red blood cell. NDV: Newcastle disease virus; dpi: days post inoculation; IBV: infectious bronchitis virus; IBD: infectious bursal disease virus; SE: standard error.
[2]In LPC-supplemented diets (+), emulsifier product (Lipidol, Easy Bio Co., Gangnam-gu, Seoul, Korea) was supplemented to the diet at the level of 0.1%.
[a,b]Means with no common superscripts within the column of each classification (fat sources or LPC) are significantly ($P < 0.05$) different.

Table 6. Effect of lysophosphatidylcholine (LPC) emulsifier on antibody responses against different antigens in broiler chicks fed different fat sources (Experiment 2).[1]

		SRBC (Log_2)		NDV (Log_2)			
Fat sources	LPC[2]	Primary	Secondary	7 dpi	14 dpi	IBV (Log_{10})	IBD (Log_{10})
SO	–	3.00	2.33	3.17	3.89	3.25	3.80
	+	4.62	2.62	3.17	3.99	3.79	4.30
SFFA	–	3.75	3.12	2.44	3.05	2.93	4.22
	+	4.75	3.00	2.88	3.25	3.22	4.51
PFP	–	2.16	2.37	2.72	3.49	3.40	3.20
	+	4.37	3.50	3.44	4.37	3.64	3.92
SE		1.58	0.87	0.71	0.62	0.52	0.61
Fat sources							
SO		3.92	2.50	3.17	3.94[a]	3.52	4.05[a,b]
SFFA		4.25	3.06	2.66	3.15[b]	3.08	4.36[a]
PFP		3.42	2.93	3.08	3.93[a]	3.52	3.56[b]
SE		1.23	0.63	0.59	0.40	0.37	0.39
LPC							
–		3.05	2.63	2.77	3.48	3.19	3.74[b]
+		4.58	3.04	3.17	3.87	3.55	4.24[a]
SE		1.00	0.54	0.45	0.36	0.33	0.28
Probability							
Fat sources		0.6523	0.5664	0.4958	0.0412	0.3322	0.0338
LPC		0.0808	0.3540	0.3014	0.1905	0.2103	0.0420
Fat sources × LPC		0.8471	0.5124	0.7247	0.5051	0.8962	0.7517

[1]SO: soy oil; SFFA: soy free fatty acids; PFP: palm fat powder; SRBC: sheep red blood cell. NDV: Newcastle disease virus; dpi: days post inoculation; IBV: infectious bronchitis virus; IBD: infectious bursal disease virus; SE: standard error.

[2]In LPC-supplemented diets (+), emulsifier product (Lipidol, Easy Bio Co., Gangnam-gu, Seoul, Korea) was supplemented to the diet at the level of 0.1%.

[a,b]Means with no common superscripts within the column of each classification (fat sources or LPC) are significantly ($P < 0.05$) different.

Table 7. Effect of lysophosphatidylcholine (LPC) emulsifier on ileal nutrient digestibility (%) in broiler chicks fed different fat sources (Experiments 1 and 2).[1]

		Experiment 1				Experiment 2			
Fat sources	LPC[2]	Crude protein	Ether extract	Total ash	Organic matter	Crude protein	Ether extract	Total ash	Organic matter
SO	–	75.28	74.15	65.05	71.83	71.35	73.19	65.26	69.37
	+	77.02	76.82	66.83	72.92	71.88	73.10	66.61	70.14
SFFA	–	71.29	73.35	60.52	71.78	69.77	71.15	61.80	70.19
	+	73.32	74.95	62.88	73.28	70.20	74.24	63.99	70.74
PFP	–	74.03	69.43	63.32	69.15	73.05	67.69	64.15	67.18
	+	74.27	71.88	64.28	71.67	70.81	70.39	62.85	68.78
SE		3.45	3.73	3.14	2.38	4.61	3.42	3.13	2.24
Fat sources									
SO		76.15	75.48[a]	65.94[a]	72.37	71.61	73.15[a]	65.93	69.76
SFFA		72.30	74.15[a,b]	61.70[b]	72.53	69.98	72.69[a]	62.89	70.46
PFP		74.15	70.65[b]	63.80[a,b]	70.41	71.93	69.04[b]	63.50	67.98
SE		2.73	2.36	1.92	1.66	3.94	1.81	2.85	1.70
LPC									
–		73.53	72.31	62.96	70.92	71.39	70.67	63.74	68.91
+		74.87	74.55	64.66	72.62	70.96	72.58	64.48	69.89
SE		2.49	1.69	1.40	1.41	3.09	1.57	2.09	1.35
Probability									
Fat sources		0.3266	0.0290	0.0245	0.2726	0.7985	0.0341	0.3303	0.1959
LPC		0.5183	0.1226	0.1544	0.1569	0.8680	0.1554	0.6673	0.3886
Fat sources × LPC		0.9290	0.9457	0.8829	0.8755	0.8794	0.5556	0.6889	0.9200

[1]SO: soy oil; SFFA: soy free fatty acids; PFP: palm fat powder; SE: standard error.

[2]In LPC-supplemented diets (+), emulsifier product (Lipidol, Easy Bio Co., Gangnam-gu, Seoul, Korea) was supplemented to the diet at the level of 0.1%.

[a,b]Means with no common superscripts within the column of fat sources are significantly ($P < 0.05$) different.

DISCUSSION

It has been reported that supplementing an exogenous emulsifier to corn-based diets can improve performance in broiler chicks (Jones et al., 1992; Zhang et al., 2010). In the present studies, dietary inclusion of LPC improved ADWG (in Exp. 1) and FCR values (in both trials). Feed conversion ratio was improved by 5.9% as the result of LPC supplementation in Exp. 1. On the other hand, there was no marked difference between dietary fat sources for ADWG. These results agree with the findings of Vieira et al. (2002), who added 4% soybean soapstock to broiler diets and did not observe any difference in weight gains. Similarly, Sanz et al. (2000) and Viveros et al. (2009) found no effect of dietary fat source on performance parameters of broiler chickens.

Although ADWG and FCR were not influenced by supplemental fat sources, ADFI was greater in birds fed diets containing PFP compared to those fed SO or SFFA diets. These results disagree with the findings of Velasco et al. (2010), who found better FCR values in broiler chickens fed diets containing sunflower oil compared with those fed palm oil diets. Several researchers (Zollitsch et al., 1997; Crespo and Esteve-Garcia, 2001) observed improvements in FCR values of broilers with a degree of unsaturation of dietary fat source, which was likely attributed to the higher digestibility of UFA than that of SFA.

Birds fed LPC-supplemented diets revealed the greater ADWG and ADFI when SFFA was included in the diets, suggesting that the energy of diets containing higher FFA content was not utilized as efficiently as the energy in diets with lower FFA content. In this regard, many fat-related factors have been shown to affect the utilization of supplemental dietary fat sources, including FFA content, the degree of saturation of fatty acids, the chain length of the fatty acids, and the positional effects of fatty acids on a triglyceride molecule (Leeson and Summers, 2001).

As noted in Exp. 2, ADWG was significantly greater in the birds fed diets containing SO when compared with those on PFP diets. This observation is in agreement with the findings of Thacker et al. (1994) and Zollitsch et al. (1997), who reported similar or lower weight gains in birds fed saturated fats than in birds fed diets containing unsaturated fat sources. It has been shown that the anti-nutritional effect of wheat arabinoxylans amplifies the digestibility differences among different fat sources (Choct et al., 1996). Water-soluble arabinoxylans of wheat have been shown to increase intestinal viscosity and exert anti-nutritive effects (Bedford and Classen, 1992). Smulikowska (1998) suggested that an increased intestinal viscosity might lead to reduced gut motility, which, in turn, may decrease the rate of diffusion and the convective transportation of emulsion droplets, fatty acids, mixed micelles, bile salts, and lipase within the small intestine. In contrast to Exp. 1, LPC supplementation of wheat diets did not improve ADWG, but improved FCR values by 2.1%. Dietary LPC inclusion was more effective in PFP diets, indicating that wheat NSP have more negative impact on digestibility and utilization of saturated fat sources (i.e., PFP; see Table 2) compared with unsaturated ones. The lack of ADWG response to supplemental LPC in Exp. 2 is largely because of the lower inclusion rates of wheat into the basal experimental diets. In other words, the level of soluble fiber (coming from wheat) and subsequently its negative impact is minor enough that supplemental LPC could manifest a considerable effect on performance parameters in Exp. 2.

In Exp. 1, the bursa weight was significantly greater in birds fed diets supplemented with LPC than those fed unsupplemented diets. In addition, dietary LPC supplementation increased SRBC antibody titer. Consistent with our findings, Cho et al. (2012) reported that broilers supplemented with sodium stearoyl-2-lactylate (as an emulsifier source) had heavier bursa of Fabricius than those fed unsupplemented diets. As shown, PFP increased IBV and SRBC antibody titers. It seems that the greater antibody responses in birds fed PFP diets is related to the lower weight gains in this group, because the reports show that there is a negative correlation between weight gain and immunological functions (Parmentier et al., 1997, 1998; Li et al., 2000).

As observed in Table 4, the relative thymus weights of birds fed SO or SFFA diets were significantly greater than those fed PFP-supplemented diets in Exp. 2. Dietary supplementation of LPC increased IBD antibody titer. On the other hand, inclusion of SFFA in the diets decreased antibody response to NDV. The exact reason for this observation remains to be elucidated; however, a probable reason for antibody depression as the result of dietary inclusion of SFFA may be the higher peroxide values of this fat source (Table 2). It has been well documented that dietary fat sources with higher peroxide values (or oxidized fat sources) suppress antibody responses in different poultry species (Dibner et al., 1996; Laika and Jahanian, 2015).

Ileal digestibility of EE was greater in birds fed diets containing SO compared with those on PFP-diets in Exp. 1. This decreased EE digestibility may be responsible for the lower weight gains (Table 3) seen in the birds fed PFP diets. On the other hand, ash digestibility was greater in the birds fed diets containing SO than birds fed SFFA diets. Probably, FFA content of this fat source (Table 2) is responsible for this observation, because previous findings have shown that FFA could bind calcium and decrease its bioavailability for young chicks (Nir et al., 1993; Noy and Sklan, 1995) and laying hens (Jahanian and Rasouli, 2014b). Although not significant, dietary LPC supplementation caused numerical increases in digestibility coefficients of EE, total ash, and organic matter. Consistent with the present findings, Gomez and Polin (1976) observed the better fat absorption when bile salts were added into the diets. Similarly, Maisonnier et al. (2003) and

Abbas et al. (2016) observed that dietary supplementation of bile salts or lecithin, respectively, increased fat digestibility in broiler chicks. Freeman (1984) mentioned that bile salts emulsify fats, creating smaller lipid droplets, and thereby could enhance the lipase action. It may be concluded that LPC increases the availability of smaller lipid droplets for lipase action, consequently improving EE digestibility (Table 7).

Similar to Exp. 1, the coefficient of ileal EE digestibility was significantly affected by the dietary fat source in Exp. 2. Ether extract digestibility was greater in birds fed SO or SFFA diets compared with those fed PFP-supplemented diets. The digestibility of fats is limited in younger chickens, as the lipase they secrete is not enough. Although some published data indicate that the daily net duodenal secretion of lipase increases 20-fold with age (Noy and Sklan, 1995), the secretion of lipase when calculated per gram of consumed feed is less dramatic. This indicates that the lipase secretion of younger birds may not be as inadequate as expected when their feed intake is taken into account (Meng et al., 2004). Bile salts also play an essential role in the digestion of lipid; however, their secretion is considered to be the principal limitation for lipid utilization during the first weeks after hatch (Jin et al., 1998; Leeson and Summers, 2001). Soy oil is primarily composed of UFA (Table 2), which are easier to digest than SFA, especially in young birds. Birds younger than 2 wk secrete limited amounts of bile acids (Krogdahl, 1985), which might impair fat digestion, especially with saturated fat sources. Hence, the lack of sufficient production of bile salts in chickens may account for the lower EE digestibility observed in birds fed PFP than those fed SO. Hetland et al. (2003) found that the inclusion of additional fiber in the diet increased the concentration of bile acids in the chyme of broilers. Therefore, fiber inclusion could improve fat digestibility more in diets based on PFP than in those based on SO.

Crude protein digestibility was not affected in Exp. 2. Despite the fact that NSP may directly decrease the digestibility of CP and amino acids, there is sound evidence that wheat pentosans increase the secretion of endogenous amino acids in broiler chicks (Angkanaporn et al., 1994). It seems that the coarse feed particles, such as those provided by the fibrous feed ingredients (like wheat diets) remain longer in the upper parts of the gastrointestinal tract, stimulating gizzard activity (Hetland et al., 2005) and increasing the production of HCl (Denbow, 2000). This is a probable reason that CP digestibility was not negatively affected by dietary inclusion of wheat.

CONCLUSIONS

The present findings show that dietary LPC supplementation improved ADWG (by 4.6%) only in corn/soybean meal-based diets. In addition, supplemental LPC increased antibody titers against SRBC and NDV in corn/soybean meal-based diets and IBD antibody response in wheat-containing diets. The greatest ADWG was allotted to the birds fed SO diets and supplemental LPC increased ADWG to a greater extent in SFFA diets. Ileal EE digestibility was greater in birds fed diets containing SO, and birds fed SFFA diets showed similar ADWG and FCR as those of SO-supplemented chicks. From the present observation, it seems that recycled fat sources (such as SFFA) can be used in poultry diets with a good efficiency to reduce feed costs, because of their lower price.

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