

« Research Note »

Effects of Exogenous Lysolecithin Emulsifier Supplementation on the Growth Performance, Nutrient Digestibility, and Blood Lipid Profiles of Broiler Chickens

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This study aimed to evaluate the effects of dietary supplementation of lysolecithin emulsifier on growth performance, nutrient digestibility, and blood lipid profiles in growing broiler chickens. In total, 1,020 1-day-old male Ross 308 broiler chickens with an average initial live weight of 43 ± 1.2 g were randomly allotted to five dietary treatments for a 35 d experiment. The treatments included: (1) NC, negative control (metabolizable energy (ME) = 3,100 kcal/kg for phase 1 and phase 2), (2) PC, positive control (ME = 3,200 kcal/kg), (3) T1, NC + 0.03% lysolecithin, (4) T2, NC + 0.06% lysolecithin, and (5) T3, NC + 0.09% lysolecithin. During days 1–35, the feed conversion ratios (FCR) of broiler chickens fed with T2 and T3 diets were lower than those of broiler chickens fed with NC diet ($P < 0.05$). On day 35, the total tract nutrient retention (TTNR) of gross energy and ether extract of broiler chickens fed with PC and T3 diets was higher than those fed with NC diet ($P < 0.05$). However, serum total cholesterol, triglyceride, and free fatty acid levels were not influenced by lysolecithin supplementation. In conclusion, lysolecithin supplementation improved FCR and TTNR of energy and ether extract when broiler chickens were offered a reduced energy diet.

Key words: blood/lipid profiles, broiler chickens, digestibility, lysolecithin, performance

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Introduction

Diet energy density is one of the important factors contributing to the rapid growth of broiler chickens in a short time span. Animal fat and vegetable oil are the main supplements that are usually added to poultry diets for increasing energy concentration and improving growth performance (Blanch *et al.*, 1996). Bile salts act as emulsifiers that disperse fat into small droplets in an aqueous environment after dietary fat enters the gastrointestinal tract. The emulsified fats are hydrolyzed by lipase and the products aggregate with bile salts to form micelles. However, particularly in young birds, the digestive tract is not developed sufficiently to produce and secrete bile salts and lipase, and the absorption and digestion of high levels of dietary lipids is inefficient (Noy and Sklan, 1998; Al-Marzooqi and Leeson, 1999). Therefore, exogenous emulsifiers may be suitable alternatives for overcoming the problems associated with high fat diet and low bile acid excretion. Several previous studies

have reported that exogenous emulsifiers increased the growth performance of broiler chickens (Melegy *et al.*, 2010; Guerreiro Neto *et al.*, 2011; Zhang *et al.*, 2011).

Lysolecithin also called lysophosphatidylcholine, is formed by the hydrolysis of phospholipids and is known to be an effective emulsifier in the food industry (Van Nieuwenhuyzen, 1981). The addition of lysolecithin to diet effectively decreased the size of fat globules and increased the active surface of fats for enzymatic digestion (Gu and Li, 2003; Gheisar *et al.*, 2015). This is because fat globules are not easily enzymatically digested and persist as indigestible residues within the intestinal tract. Lysolecithin feeding was recently reported to increase egg weight and feed efficiency in laying hens (Han *et al.*, 2010), and improve growth performance of broiler chickens in the starter period due to increased fatty acid digestibility (Zhang *et al.*, 2011). In addition, dietary emulsifier supplementation increased lipid absorption and decreased the free fatty acid and cholesterol levels in blood (Jones *et al.*, 1992). We hypothesized that lysolecithin might promote the emulsification process *in vivo* and assist in the efficient digestion of fats. However, studies assessing the effects of various emulsifiers in broiler chickens are limited and inconsistent. Therefore, the objective of this study was to determine the effects of lysolecithin on

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growth performance and nutrient digestibility of broiler chickens fed with two dietary levels of metabolizable energy (ME).

Materials and Methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Institutional Animal Care and Use Committee of Dankook University (IACUC protocol No. DKU-16-025).

Experimental Design

In total, 1,020 male broiler chickens (Ross 308) with an average body weight of 43 ± 1.2 g were randomly assigned to five treatment groups with 17 birds/cage and 12 cages/treatment for a 35-day experiment. The birds were fed diets based on corn and soybean meal in two phases: days 1 to 21 and days 22 to 35 (Table 1). The negative control (NC) diets were formulated to contain 100 kcal/kg less energy than the positive control (PC), based on a previous study (Park and Kim, 2016) that showed significant differences in bird production and blood lipid metabolites. The treatments included: (1) NC (ME=3,100 kcal/kg), (2) PC (ME=3,200 kcal/kg), (3) T1, NC+0.03% lysolecithin, (4) T2, NC+

0.06% lysolecithin, and (5) T3, NC+0.09% lysolecithin. All broiler chickens were allowed *ad libitum* access to feed and water throughout the experiment, and were housed in an environmentally controlled room. Temperature was gradually reduced from 33°C on day 1 to 23°C by the end of the experiment.

Experimental Procedures and Sampling

Body weight and feed intake per cage were recorded from day 1 to 21 of age and day 22 to 35 of age, respectively, and used to calculate body weight gain, feed intake, and feed conversion ratio. At day 32, 0.2% chromium oxide was added to the diet as an indigestible marker for three days before fecal collection to determine total tract nutrient retention (TTNR) of dry matter, nitrogen, ether extract, and gross energy (Fenton and Fenton, 1979). On day 35, fecal samples of 12 replicates from each treatment were collected for TTNR analysis. For blood profiles, 24 birds per treatment (two birds per cage) were randomly selected and blood samples were taken from a vein in the left wing. All blood and fecal samples were stored at -20°C until analysis.

Laboratory Analysis

Before chemical analysis, fecal samples were dried at 60°C

Table 1. Composition of experimental diets (as-fed basis)

Ingredients (%)	Phase 1 ¹		Phase 2	
	NC	PC	NC	PC
Corn	53.58	51.71	59.15	57.27
Soybean meal	25.14	24.09	19.65	18.59
Corn gluten meal	9.66	10.54	5.25	6.13
Canola meal	5.00	5.00	10.00	10.00
Tallow	3.00	5.00	3.00	5.00
Tricalcium phosphate	1.81	1.84	1.35	1.36
Limestone	0.84	0.84	0.93	0.92
Salt	0.46	0.46	0.34	0.34
DL-Methionine (98%)	0.16	0.17	0.05	0.05
L-Lysine (99%)	0.04	0.07	0.001	0.03
Vitamin premix ²	0.15	0.15	0.15	0.15
Mineral premix ³	0.15	0.15	0.15	0.15
Calculated composition				
ME (kcal/kg)	3,100	3,200	3,100	3,200
CP (%)	23.0	23.0	20.0	20.0
Lysine (%)	1.10	1.10	1.00	1.00
Methionine (%)	0.50	0.50	0.38	0.38
Ca (%)	1.00	1.00	0.90	0.90
Available P (%)	0.76	0.76	0.68	0.68
Crude fat (%)	5.47	7.36	5.56	7.45
Crude fiber (%)	3.12	3.04	3.41	3.34
Analyzed composition				
GE (kcal/kg)	3,750	3,860	3,740	3,880
CP (%)	22.5	22.8	19.4	20.2
Crude fat (%)	5.38	7.41	5.44	7.40

¹Phase 1 diet provided during days 1 to 21; Phase 2 diet provided during days 22 to 35.

²Provided per kg feed: 15,000 IU vitamin A, 3,750 IU vitamin D3, 37.5 mg vitamin E, 2.55 mg vitamin K3, 3 mg thiamin, 7.5 mg riboflavin, 4.5 mg vitamin B6, 24 µg vitamin B12, 51 mg niacin, 1.5 mg folic acid, 0.2 mg biotin, and 13.5 mg pantothenic acid.

³Provided per kg feed: 37.5 mg Zn, 37.5 mg Mn, 37.5 mg Fe, 3.75 mg Cu, 0.83 mg I, 62.5 mg S, and 0.23 mg Se.

for 72 h, after which they were ground and passed through a 1-mm screen. Feed and fecal samples were analyzed for dry matter (Method 930.15; AOAC, 2007), crude protein (Method 990.03; AOAC, 2007), and ether extract (Method 960.39; AOAC, 2007). Chromium content was analyzed using ultraviolet (UV) absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan). The gross energy values of the diet and excreta samples were measured by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL, USA). The total tract nutrient retention (TTNR) was calculated using the following formula:

$$\text{TTNR (\%)} = \{100 - (\text{marker in feed} / \text{nutrient in feces}) \times (\text{marker in feces} / \text{nutrient in feed})\}$$

Blood samples collected for lipid measurements in non-heparinized vacuum tubes were centrifuged ($3,000 \times g$) at 4°C for 15 min. The total cholesterol and triglyceride levels in the serum samples were analyzed using an auto analyzer (Automatic Biochemical Analyzer, RA-1000; Bayer Corp., Tarrytown, NY, USA) and colorimetric methods. The free fatty acid content in serum was measured using commercial test kits according to the manufacturer's instructions (Enzychrom, Bio-Assays Systems, CA, USA).

Statistical Analysis

The replicates were the experimental units for evaluation of growth performance and total tract nutrient retention. Each broiler was the experimental unit for blood lipid metabolite determination. Data were analyzed using the GLM procedure of SAS as a randomized complete block design (SAS Inst. Inc., Cary, NC, USA). Tukey's post hoc test was performed to detect the significance of differences among groups. The statistical difference of the values was expressed at $P < 0.05$ and results were expressed as means \pm standard error of the mean (SEM).

Results and Discussion

The primary objective of this study was to investigate the influence of lysolecithin supplementation and different dietary levels of ME on nutrient digestibility and blood lipid profiles in broiler chickens, and their relationship with productivity.

During phase 1 and phase 2 of this study, tallow and fat content in the PC diet was about 2% higher than that in the NC diet. As a result, broiler chickens fed with PC diet during days 1–35 showed a significant difference in the feed conversion ratio ($P < 0.05$), whereas no difference was observed in weight gain and feed intake between the NC and PC groups (Table 2). The feed conversion ratio in broiler chickens fed with T2 and T3 diets containing 0.06% and 0.09% lysolecithin, respectively, improved during day 1–35 compared to those fed with NC diet ($P < 0.05$). However, lysolecithin supplementation did not affect weight gain and feed intake or feed conversion ratio during days 1–21, 22–35, or 1–35.

Previous studies indicated that the emulsifier improved homogenization of the ingesta, which may result in enhanced utilization efficiency of dietary fat and improved growth

performance and lipid metabolism in broiler chickens and pigs (Dierick and Decuypere, 2004). Although physiological roles of emulsifying activity have been reported, its effects on productivity remain controversial. For example, Melegy *et al.* (2010), Roy *et al.* (2010), and Zhang *et al.* (2011) reported improvements in body weight or feed efficiency with the addition of emulsifiers to broiler chicken feeds. In addition, Overland *et al.* (1993) suggested that the effect of emulsifiers on growth performance might be more beneficial when animal fats are used. In contrast, Azman and Ciftci (2004) reported that emulsifiers did not have any significant effect on the productivity of broiler chickens in terms of body weight and feed conversion ratio. Overland *et al.* (1993, 1994) did not observe any benefit in the growth performance of weanling or growing-finishing pigs using soy lecithin. The inconsistent results regarding growth performance could be due to differences in the types and levels of dietary fat sources (soybean oil, palm oil, tallow, poultry fat) and the major component of emulsifying agents (soybean lecithin, lysophosphatidylcholine, glyceryl-polyethylene glycol ricinoleate). Moreover, the results of the present study were similar to those of Khonyoung *et al.* (2015) reported that no statistical difference occurred in bird body weight gain, but improvement in feed conversion ratio between emulsifier and control treatments was observed. The findings of the present study suggest that the addition of lysolecithin emulsifier to low energy diets alleviated the negative effects and improved feed conversion ratio.

The results presented in Table 3 indicate that supplementing low energy level diets with 0.09% lysolecithin significantly enhanced digestibility of energy and ether extract, and demonstrate that reducing the dietary energy levels in NC diet had a negative impact on the digestibility of energy and ether extract ($P < 0.05$); however, emulsifier supplementation (0.09%) of NC diet (low energy diet) improved ($P < 0.05$) the digestibility of energy and ether extract, which were equal to those of the PC treatment (high energy diet). Our observations are supported by the results of Guerreiro Neto *et al.* (2011), Cho *et al.* (2012), and Abbas *et al.* (2016), who reported that the addition of emulsifier to diet increased the digestibility of ether extract in broiler chickens. Feeding broiler chickens with dietary emulsifier in the late growth phase has also been reported to enhance energy digestibility (Gheisar *et al.*, 2015). Exogenous emulsifier supplementation reportedly improved fat utilization in young chickens because low lipase levels limit the digestion and intestinal absorption of fat in young birds (Al-Marzooqi and Leeson, 1999). This might be attributed to the action of lysolecithin as a dietary fat emulsifier, which, along with increased lipolysis of triglycerides, results in higher levels of micelle formation, digestion, and absorption of fats (Cho *et al.*, 2012). The present study suggests that the lysolecithin addition to low energy diets alleviated the negative effects and improved the feed conversion ratio by increasing feed digestibility. However, addition of the lysolecithin increased the TTNR of ether extract and energy, although this effect was not translated into improvements in weight gain.

Table 2. The effects of diet ME and lysolecithin supplementation on growth performance in broiler chickens^{1,2}

Item	NC	PC	T1	T2	T3	SEM ³	P value
Day 1-21							
Weight gain, g	712	743	727	727	732	4.17	0.246
Feed intake, g	1016	1053	1051	1043	1046	4.16	0.958
Feed conversion ratio	1.469	1.419	1.450	1.436	1.433	0.01	0.509
Day 22-35							
Weight gain, g	985	1030	1010	1025	1030	10.05	0.606
Feed intake, g	1755	1750	1740	1735	1743	10.07	0.976
Feed conversion ratio	1.783	1.701	1.724	1.700	1.703	0.01	0.133
Day 1-35							
Weight gain, g	1698	1773	1737	1752	1762	10.04	0.154
Feed intake, g	2801	2802	2791	2778	2789	12.27	0.973
Feed conversion ratio	1.650 ^a	1.582 ^b	1.607 ^{ab}	1.587 ^b	1.585 ^b	0.01	0.003

¹ NC, negative control (ME=3,100 kcal/kg for phase 1 and phase 2; PC, positive control (ME=3,200 kcal/kg); T1, NC+0.03% lysolecithin; T2, NC+0.06% lysolecithin; T3, NC+0.09% lysolecithin.

² Values represent the mean of 12 replicates.

³ Standard error of the mean.

^{a,b} Means in the same row with different superscripts differ significantly ($P < 0.05$).

Table 3. The effects of diet ME and lysolecithin supplementation on total tract nutrient retention of dry matter, nitrogen, energy and ether extract in broiler chickens^{1,2}

Item	NC	PC	T1	T2	T3	SEM ³	P value
Day 35							
Dry matter, %	70.5	71.9	70.7	70.9	71.4	0.45	0.876
Nitrogen, %	67.8	69.5	68.1	68.3	68.7	0.44	0.803
Energy, %	68.5 ^b	70.5 ^a	69.6 ^{ab}	69.5 ^{ab}	70.2 ^a	0.51	0.048
Ether extract, %	79.5 ^b	81.8 ^a	80.5 ^{ab}	81.5 ^{ab}	81.3 ^a	0.29	0.045

¹ NC, negative control (ME=3,100 kcal/kg for phase 1 and phase 2; PC, positive control (ME=3,200 kcal/kg); T1, NC+0.03% lysolecithin; T2, NC+0.06% lysolecithin; T3, NC+0.09% lysolecithin.

² Values represent the mean of 12 replicates.

³ Standard error of the mean.

^{a,b} Means in the same row with different superscripts differ significantly ($P < 0.05$).

Table 4. The effects of diet ME and lysolecithin supplementation on serum total cholesterol, triglyceride and free fatty acid levels in broiler chickens^{1,2}

Item	NC	PC	T1	T2	T3	SEM ³	P value
Day 35							
Total cholesterol, mg/dL	140	158	144	149	150	2.24	0.115
Triglyceride, mg/dL	40.2	46.3	42.1	41.7	43.8	1.08	0.443
Free fatty acid, uEq/L	428	440	432	444	431	7.21	0.950

¹ NC, negative control (ME=3,100 kcal/kg for phase 1 and phase 2; PC, positive control (ME=3,200 kcal/kg); T1, NC+0.03% lysolecithin; T2, NC+0.06% lysolecithin; T3, NC+0.09% lysolecithin.

² Values represent the mean of 12 replicates.

³ Standard error of the mean.

In the present study, inclusion of lysolecithin did not affect serum total cholesterol, triglyceride, or free fatty acid concentrations (Table 4). Previous studies on the effect of emulsifiers on blood lipid profiles of broiler chickens are limited. In addition, the results of studies on the response of lipid metabolites to dietary emulsifier supplementation are inconsistent. Roy *et al.* (2010) and Guerreiro Neto *et al.* (2011)

reported that serum total cholesterol, triglyceride, and HDL-cholesterol levels of broiler chicken fed with diets containing vegetable oil or animal fat were not affected by emulsifier supplementation (glyceryl polyethylene glycol ricinoleate and caseinate, respectively). Conversely, Cho *et al.* (2012) reported that broiler chickens fed with diet containing 0.05% emulsifier (sodium stearoyl-2-lactylate) had lower serum

triglycerides than those fed with high-energy diets without emulsifier. We presume that the effect on lipid metabolites may depend on fat sources and emulsifier types. The use of emulsifiers in modulating lipid metabolism in poultry needs to be further investigated.

In conclusion, the current study indicates that reduction in dietary energy levels adversely affected the feed conversion ratio of broiler chickens; however, supplementation of low energy diets with emulsifiers could alleviate these negative effects and improve the feed conversion ratio. The inclusion of an emulsifier in broiler chickens diet also improved the TTNR of energy and ether extract.

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