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Effect of Lysophospholipids Supplementation on Growth Performance, Nutrient Digestibility, Blood Profiles and Carcass Traits in Broilers

사료 내 Lysophospholipids의 첨가가 육계의 성장성적, 영양소 소화율, 혈액 성상 및 도체특성에 미치는 영향

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By
Hyun, Yoon Kyung

School of Agricultural Biotechnology
Graduate School of Seoul National University

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지도교수 **김 유 용**

이 논문을 농학석사 학위논문으로 제출함

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현 윤 경

현윤경의 농학석사 학위논문을 인준함

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Summary

Present broilers has been genetically modified to grow fast and their nutrient requirements is also increased. While nutrients utilization in broiler is limited especially in young age, considerable amount of nutrients are excreted. This loss cause not only environment problem but also ascending feed cost. So it is needed to improve absorption of nutrients.

This study was conducted to evaluate effects of dietary lysophospholipids supplementation to low-energy diet on growth performance, nutrient digestibility, blood profiles, and carcass traits in broilers. A total of 300 male (Ross308®) day-old broilers, with an average initial body weight of 47.0 g were allotted to 1 of 5 dietary treatments with 6 replicates and 10 chicks per pen in randomized complete block (RCB) design. The five dietary treatments were : 1) positive control (PC; T1): the diet was 3,025 (starter period), 3,150 (grower period), and 3,200 kcal/kg (finisher period) of metabolizable energy (ME) that recommended by the breeding company; 2) negative control (NC; T2): the diets contained less 150kcal/kg of ME than PC treatment; 3) T3: NC + lysophospholipids 0.05%; 4) T4: NC + lysophospholipids 0.10%; 5) T5: NC + lysophospholipids 0.15%. Results revealed that the birds fed with lysophospholipids had greater body weight (BW), body weight gain (BWG) and better feed conversion ratio (FCR) during grower and finisher periods than that of birds in NC

(P<0.01). The supplementation of lysophospholipids showed linear responses to BW, BWG and FCR during grower, finisher and overall periods (P<0.01). But no significant difference was detected in feed intake (FI) throughout the entire experimental periods (P>0.05). Supplementation of lysophospholipids had a positive effect on digestibility of dry matter (P<0.05), crude protein (P<0.01) and fat (P=0.08) compared with NC. Especially dry matter and crude protein digestibility had linear response (P<0.01) by lysophospholipids addition whereas digestibility of amino acids had no significant difference among treatments. The relative organ weights of abdominal fat, bursa of fabricius, and spleen of birds were not affected by dietary treatments, however, the relative weight of breast muscle was increased with lysophospholipids inclusion (P<0.05). Moreover, at the level of 0.15% lysophospholipids, the relative weight of liver was decreased compared to NC (P<0.05). No significant differences were observed in aspartate transaminase, total cholesterol, triglyceride, low density lipoprotein cholesterol and high density lipoprotein cholesterol among treatments. Consequently, dietary lysophospholipids could be used to low energy diet of broilers in order to improve growth performance, feed efficiency, carcass compositions with improvement of nutrients digestibility and without any detrimental effect on lymphoid and metabolic function of organ.

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Ⅰ. Literature Review

List of Abbreviations

AME	: Apparent metabolizable energy
AOAC	: Association of official analytical chemists
AST	: Aspartate transaminase
BW	: Body weight
BWG	: Body weight gain
CMC	: Critical micelle concentration
CP	: Crude protein
DM	: Dry matter
EE	: Ether extract
FCR	: Feed conversion ratio
HDL	: High density lipoprotein
HLB	: Hydrophilic lipophilic balance
ME	: Metabolizable energy
LDL	: Low density lipoprotein
TC	: Total cholesterol

I. Introduction

World chicken meat consumption has been grown to 93.4 million tons in 2013 with about 3.7% of annual growing rate in recent 10years. And this growing trend is expected to continue as world population and chicken meat consumption per person predicted to grow as economic standard of developing countries is improved. Therefore broiler industry has been developing dramatically and breeding companies have been generating large and fast growing broiler. Arthur and Albers (2003) reported that daily body weight gain of broiler increased rapidly from 22 g in 1960 to 50 g in 2000. Thus nutrient requirements also has been increased to fulfill their high growth potential. And high energetic ingredients are widely used in broiler feed. Animal fats and some vegetable oils are well-established as the excellent energy yielding ingredients to improve productivity (Blanch et al., 1996). But due to limitation of broiler's digestibility nutrients are not fully absorbed and the rest are excreted. This loss of nutrients cause not only environment problem but also ascending feed cost. Especially global price of grain that major feed ingredients has been increasing due to growth of population, meat consumption and demand for biofuel industry, it is needed to improve absorption of fats as well as other nutrients.

Lysophospholipids are made by enzymatic hydrolysis of phospholipids that are widely used to food as emulsifier occur in

nature. Phospholipids molecular structure is very analogue to triglycerides but have only two fatty acids esterified to glycerol and the third position of the glycerol moiety is phosphorylated. By removing one of two fatty acids of phospholipids, lysophospholipids is more hydrophilic and more efficiently disperse lipid globule into the aqueous environment like gastrointestinal tract (Mine et al., 1992).

Researches investigated effect of dietary lysophospholipids supplementation to broiler and found it could enhance growth performance and improve nutrients digestibility (Schwarzer and Adams, 1996; Y.G. Liu. 1997; Melegy et al., 2010; Zhang et al., 2011). However, scant publications are available on low energy diet with various levels of lysophospholipids in broilers.

The aim of the present study was to evaluate effects of dietary lysophospholipids supplementation to low energy diet on growth performance, nutrient digestibility, blood profiles, and carcass traits in broilers.

Ⅱ. Review of Literature

1. Lipid in broiler diets

1.1 Digestion and absorption of lipid in broiler

Dietary lipids are mostly triglyceride form and it's two fatty acid molecules are detached during digestion, producing a monoglyceride and two free fatty acids that could be absorbed.

In poultry, digestion process is initiated by emulsification in gizzard. Feed particle size is reduced by grinding in gizzard and also duodenal contents containing bile salt and intestinal juice are penetrated by reverse passage movement (Sklan et al., 1978). And emulsified lipids are move into duodenum as triacylglycerols and phospholipids. Lipids are made into chyme by emulsification with bile salts released from gall bladder and hydrolysed by pancreatic lipase. Colipase, a low molecular weight protein secreted by the pancreas, is essential for the lipase activity. With aid of colipase, pancreatic lipase releases two fatty acids and a monoacylglycerol from triacylglycerols. And these compounds spontaneously form mixed micelles by incorporation of bile salt (Figure 1) then transported to the mucosal surface and pass through the brush border membrane (Krogdahl, 1985).

Absorption of lipids is mainly take place at the jejunum in chicken (Hurwitz et al., 1973). And the ileum is important for linoleic, stearic and palmitic acid absorption. Bile salts uptake

occur from jejunum as well as ileum by passive diffusion. It is seemed that, in poultry, lipid and bile salt absorption site is approximately same.

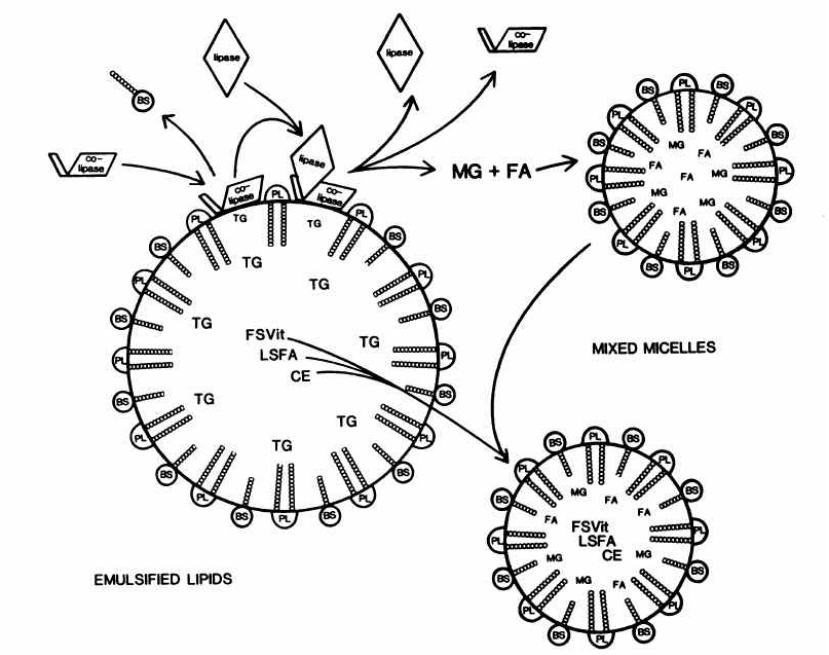

Figure 1. Sequence of events during intestinal lipolysis

(BS, bile salt; CE, Cholesteryl ester; FA, free fatty acid; FSVit, fat-soluble vitamins; LSFA, long-chain saturate fatty acids; MG, monglyceride; PL, phospholipid; TG, triglyceride)

Soure: Krogdahl (1985)

Transport of lipids through the brush border membrane is passive (Figure 2), and absorption rates is different depending on chain length and saturation degree. Short-chain fatty acids and monoglycerides are absorbed directly from the intestinal lumen to

mesentery blood vessels by passive transport (Pond et al., 2005) while long-chain saturated fatty acids, diglycerides, fat soluble vitamins and cholesteryl esters are need to be formed mixed micelles to transported to intestinal cells (Davenport, 1980).

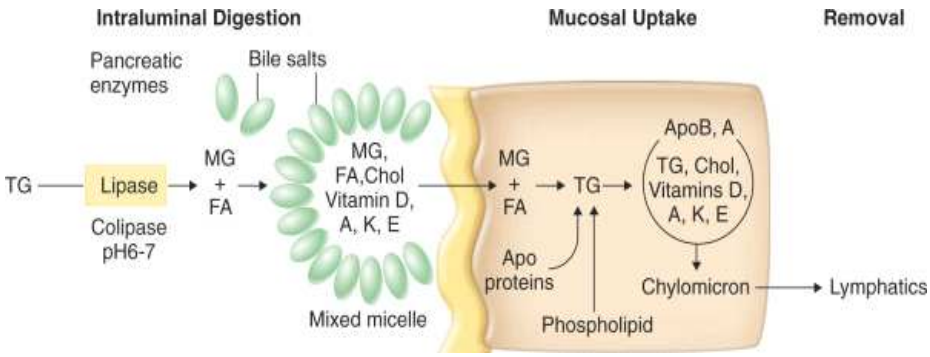

Figure 2. Absorption of dietary fat in intestinal track

(Apo proteins, apolipoproteins; Chol, cholesterol; FA, fatty acids; MG, monoglycerides; TG, triglycerides)

1.2. Factors affecting the digestibility of lipid

Digestibility of lipid is affected by many factors include age, species, source of fat, ingredients composition of diet.

In young broiler chicks, poorly developed gastrointestinal tract is considered major limiting factor of lipid digestibility and with advancing age the digestibility is increased (Renner and Hill, 1960; Carew et al., 1972, Tancharoenrat et al., 2013). Nir et al. (1993) reported relative weight of pancreas, small intestine and liver of broiler is maximized at between 5 to 10 day of age and relative activities of digestive enzymes, amylase and trypsin,

by body weight is also maximized at between 5 to 10 day of age while lipase and chymotrypsin is increasing until the end of experimental day, 15 day (Fig 3). This is coincide with study of Noy and Sklan (1995) that found secretion of lipase, trypsin and amylase increased 20 to 100 folds between 4 and 21 day, while the increasement of lipase was slowest. Nitsan et al. (1991) also found pancreatic digestive enzymes are increase to maximum level around day 10. And Krogdahl (1985) stated secretion of bile seems to be first limiting and lipase secretion or other physiological condition may be next limiting factor. Tancharoenrat et al. (2013) investigated the influence of age and fat source on total tract digestibility of lipid. As describe at Table 1, lipid digestibility is very low post hatch period and increasing by age. The reason of lower digestibility of saturated fats in young birds are not well understood but it may relate to less bile acid secretion and recirculation.

Table 1. Fat digestibility by ages and source in broiler

Fat digestibility (%)	Age of birds (week)			
	1	2	3	5
Tallow	36.8	65.3	73.6	72.6
Soy oil	59.1	89.8	96.6	94.8
Tallow : Soy oil	50.0	83.1	83.0	85.6
Poultry fat	60.0	84.5	92.8	91.1
Palm oil	60.3	80.6	83.6	84.3

Source: Tancharoenrat et al. (2013)

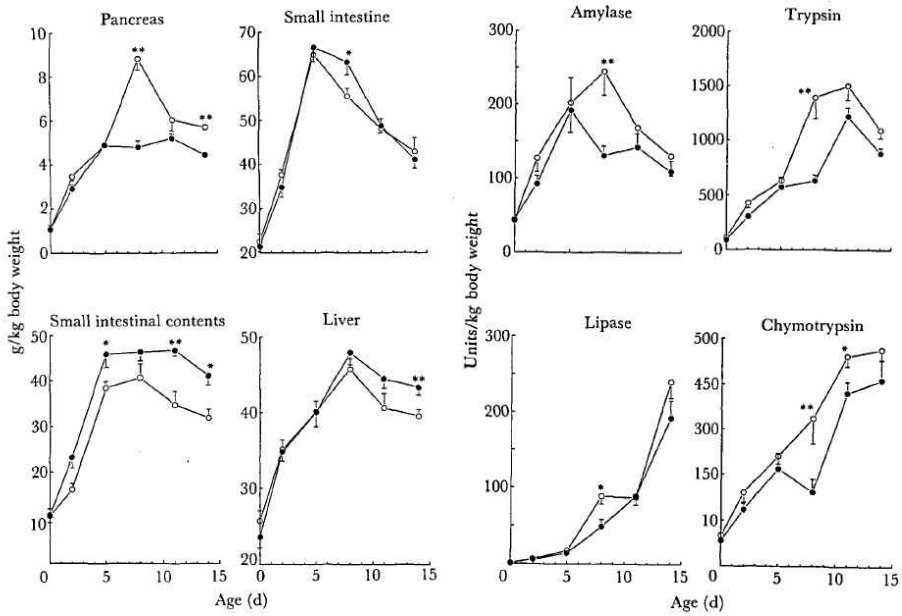

Figure 3. Relative weight of organs and enzymes secretion

(○ egg type chicks; ● broiler chicks; Vertical bars represent the SE; Values between breeds differ significantly, *P<0.05, **P<0.01)

Soure: Nir et al. (1993)

And it is well known that the degree of saturation of fatty acids has large impact on it's digestibility and also dietary fat concentration, carbon chain length, position of double bonds of fatty acids can all influence digestibility (Renner and Hill, 1961; San Juan and Villamide, 2000; Baiao and Lara, 2005). Saturated fatty acids need to be emulsified and form micelles to digestion and absorption so lipids that containing high amount of saturated fatty acids such as animal fats has lower digestibility than oils form plant (Sklan, 1979; Scott et al., 1982). Tancharoenrat et al. (2013) found soy oil that has highest unsaturated to saturated

ratio has highest digestibility and tallow that has high proportions of saturated fatty acids showed lowest digestibility.

Contents of free fatty acids is also influence to the digestibility, because it is assumed to be more easy to be peroxidated. According to Wiseman and Salvadore (1991), high level of free fatty acids reduce their metabolizable energy (ME) value and free fatty acids contents has more impact to fat that has high proportion of saturated fatty acids and fed to young birds (Table 2).

Table 2. Effect of level of free fatty acid and bird age on fat ME value (kcal/kg)

Fat source and contents of free fatty acids (%)		Age	
		10 d	54 d
Tallow	13%	7,460	7,940
	95%	4,920	6,830
Palm	6%	6,690	7,800
	92%	3,570	6,640
Soy	14%	9,290	9,300
	68%	8,000	8,480

Source: Wiseman and Salvador (1991)

Micelle helps to transportation and absorption through the microvilli of the small intestine so its formation is important for absorption of fats. And appropriate balance of saturated to unsaturated fatty acids is important due to they have an effect

on micelle formation. In the study of Atteh and Lesson (1985), the metabolizable energy of the 50:50 mixture of the unsaturated oleic acid (2920kcal/kg of ME) and saturated palmitic acid (2500kcal/kg of ME) is 5% higher than mean value (2710kcal/kg of ME) of two fatty acids' ME. Even it may not economically practical, 3:1 ratio of nusaturates:saturates is a good for optimum fat digestibility for all ages of bird.

Although it is not easily incorporated within a formulation matrix, the factors that affecting fat ME value and their relative ME value are summarized in Table 3.

Table 3. Factors affecting fat ME value

Criteria	Relative fat ME	
Bird age	28 d +	100 %
	7 - 28 d	95 %
	1 - 7 d	88 %
Free fatty acids	0 - 10 %	102 %
	10 - 20 %	100 %
	20 - 30 %	96 %
	30 % +	92 %
Inclusion level	1 %	100 %
	2 %	100 %
	3 %	98 %
	4 %	96 %
	5 % +	94 %
Calcium level	< 1%	100 %
	> 1%	96 %

Source: Leeson and Summers (2005)

Additionally non-starch polysaccharides that exist in cereal ingredients have been recognized to have anti-nutritive activity (Fengler and Marquardt, 1988; Bedford et al., 1991). And one of possible reason is to increase viscosity of ingesta. By decreasing the diffusion rete of substrates, non-starch polysaccharides interrupt the interaction with enzyme which reduces efficiency of nutrient digestion and absorption (Annison, 1993; Choct, 1997).

2. Methods for improve lipid digestibility

2.1 Various lipid source and digestibility

There are many kinds of lipid sources used in animal feed. Commonly used lipids are animal fats (lard, tallow, poultry fat, and fish oil), vegetable oils (soybean oil, palm oil, rapeseed oil, and corn oil). As mentioned before, chain length, degree of saturation, content of free fatty acid and ratio of saturates and unsaturates affected lipids digestibility. And there are various lipids that used as feed ingredient. Because their fatty acids composition is different (Table 4), digestibility is also varied. Several experiments have conducted to evaluate the effect of adding different types of lipids on fat absorption in broiler.

Pesti et al. (2002) investigated the effect of 8 fat types (feed grade poultry grease, feed grade poultry grease, restaurant grease, white grease, animal-vegetable oil blend, palm oil, yellow grease and soybean oil) growth performance and ME value.

Table 4. Fatty acid profile and ME value of lipids

Ingredient	Fatty acid profile										Metabolizable energy (kcal/kg)	
	≤10:0	12:0	14:0	16:0	18:0	16:1	18:1	18:2	18:3	≥20:4	age under 3wk	age after 3wk
Tallow			4.0	25.0	24.0	0.5	43.0	2.0	0.5		7,400	8,000
Poultry fat			1.0	20.0	4.0	5.5	41.0	25.0	1.5		8,200	9,000
Fish oil			8.0	21.0	4.0	15.0	17.2	4.4	3.0	25.0	8,600	9,000
Vegetable oil			0.5	13.0	1.0	0.5	31.0	50.0	2.0		8,800	9,200
Coconut oil	15.0	50.0	20.0	6.0	2.5	0.5	4.0	2.1	0.2		7,000	8,000
Palm oil			2.0	42.4	3.5	0.7	42.1	8.0	0.4		7,200	8,000
Restaurant grease			1.0	18.0	13.0	2.5	42.0	16.0	1.0		8,100	8,900

Source: Leeson and Summers (2005)

When birds fed diet containing 6% of different fat types, ME values were highest in soybean oil, yellow grease and animal-vegetable blend oil fed group followed by white grease and restaurant grease and lowest apparent metabolizable energy (AME) values were observed in poultry grease and palm oil fed group. But there was no difference is growth performance.

And there is several studies that reported positive effect of animal-vegetable fat blend. As mentioned above, animal fat has hight content of long chain saturated fatty acids whereas vegetable oil has high content of unsaturated fatty acids. So their blend may improve digestibility by modify the ratio of unsaturates and saturates. Sibbald (1978) reported that blend of soybean oil and tallow has higher AME than calculated value of its components. Muztar et al. (1981) observed similar result that AME of blend fat (tallow and rapeseed soapstocks) has over 4% higher than sum of the means of its components. Preston et al. (2001) also found effect of blending oil. When 3 types of fats (tallow, soybean oil or 2:1 blend of tallow to soybean oil) were supplemented to broiler diets in 6%, synergy effect were observed that the digestibility coefficient was 0.85 for soybean oil, 0.76 for blend oil and 0.69 for tallow.

In growth performance, Firman et al. (2008) observed no differences of broiler's performance when they fed diets with 3% of different fats added (soybean oil, yellow grease, poultry fat, tallow, vegetable and animal fat blend, lard and palm oil) during 7 week trial period. And Sanz et al. (2000) also found no

difference in final body weight, daily gain and feed intake when compare broiler fed sunflower oil to animal fat blend (50:50 of lard and tallow) and between tallow, lard or sunflower oil treatment group. Even the energy retention and fat retention (body fat g per 100g of weight gain) of broiler was higher in group fed animal fats (tallow, lard and 1:1 blend of lard and tallow) than thoes fed with sunflower oil.

And fat form is also important for digestibility that is extracted fat has greater digestibility compare to intact form of fat. Kil et al. (2010) investigated the digestibility of corn oil and oil in corn germ meal in growing pigs and found that extracted fat has greater digestibility than intact fat. The average apparent digestibility of extracted fat was 81.9% and that of intact fat was 63.2% and it has same tendency in true ileal digestibility. Kim et al. (2013) also reported digestibility of extracted corn oil was greater than oil in high-oil corn in growing pigs. This can be explained that intact fat is within fat cell membranes so is more hard to enzymatic digestion that extracted fat (Adams and Jensen, 1984; Bach Knudsen et al., 1993).

2.2 Effects of exogenous lipase on lipid digestibility

Because relative secretion of lipase to the body weight is not reach at peak up to 15 d of age while amylase and trypsin reach at highest level of relative secretion to the body weight at 5 d to 10 d of age (Nir et al., 1993), lipase supplementation could be helpful to increase fat digestibility.

Polin et al. (1980) found 1% of lipase addition to the diets that containing 4% of tallow increased fat absorption compared to 0 and 0.1% of addition in White Leghorn chicks. And Al-Marzooqi and Leeson (1999) conducted experiment of lipase addition in young broilers. Broilers fed diet that containing animal-vegetable fat blend (either 4% and 8%) with 0.714% of pancreatic enzyme had better fat digestibility and apparent metabolizable energy corrected for nitrogen (AMEn). And as pancreatic enzyme inclusion level increased (0, 0.214, 0.429, 0.643, 0.857, and 1.0071 %) fat digestibility and AMEn was increased. However in this study, addition of pancreatic enzyme reduced feed intake and weight gain and the authors said pancreatic enzyme contaminated with cholecystokinin may be the reason of this result. And they did another study to evaluate effect of pancreatic enzyme supplementation on gut and performance of broilers (Al-Marzooqi and Leeson, 2000). Up to 1.339% of addition, there was no effect on gut morphology and gastric motility but reduction of feed intake and weight gain is also observed with pancreatic enzyme supplementation.

But in the study of Martin and farrell (1998), there was no effect of lipase supplementation (0.023 and 0.05%) on broiler's growth performance and utilization of fat and AME that fed diet containing rice bran (20 or 40%). This may result in high fiber content of rice bran (11.4%, NRC 1994). It is well known that fat digestibility negatively affected by fiber contents especially non-starch polysaccharides that increase intestinal digesta

viscosity (Ward and Marquardt, 1983; Choct and Annison, 1992). And Tan et al. (2000) mentioned that exogenous lipase is more effective when supplemented to diets that containing more saturated fats. So effect of exogenous lipase on fat digestibility and growth performance could be varied with composition of diet ingredients.

2.3 Functions of bile salt on lipid digestibility

Bile is a bitter-tasting, dark green to yellowish brown fluid that aids the digestion of lipids in the small intestine. Bile is produced in hepatocytes and then transported to gallbladder for storage (Koeppen and Stanton, 2009). Bile is composed of water, bile pigmetns, bile salts, phospholipids, cholesterol, glycerides and some inorganic ions (Haslewood, 1978). In bile production, cholic and chenodeoxycholic aicds, the primary bile acids, are produced from cholesterol by hydrolysed with 7-α-hydroxylase. And by conjugated with taurine or glycine, these bile acis are secreted as bile salts. And after secreted to intestine, a part of primary bile acids are changes to the secondary bile acids, deoxycholic acid and lithocholic acid, by the activity of the intestinal bacteria (Figure 4).

Bile acids are steroid acids that predominate compound in the bile. Different molecular forms of bile acids can be synthesized in the liver by different species. In chicken bile, glycolithocholic acid is most abundant bile acid, followed by taurocholic acid (Table 5).

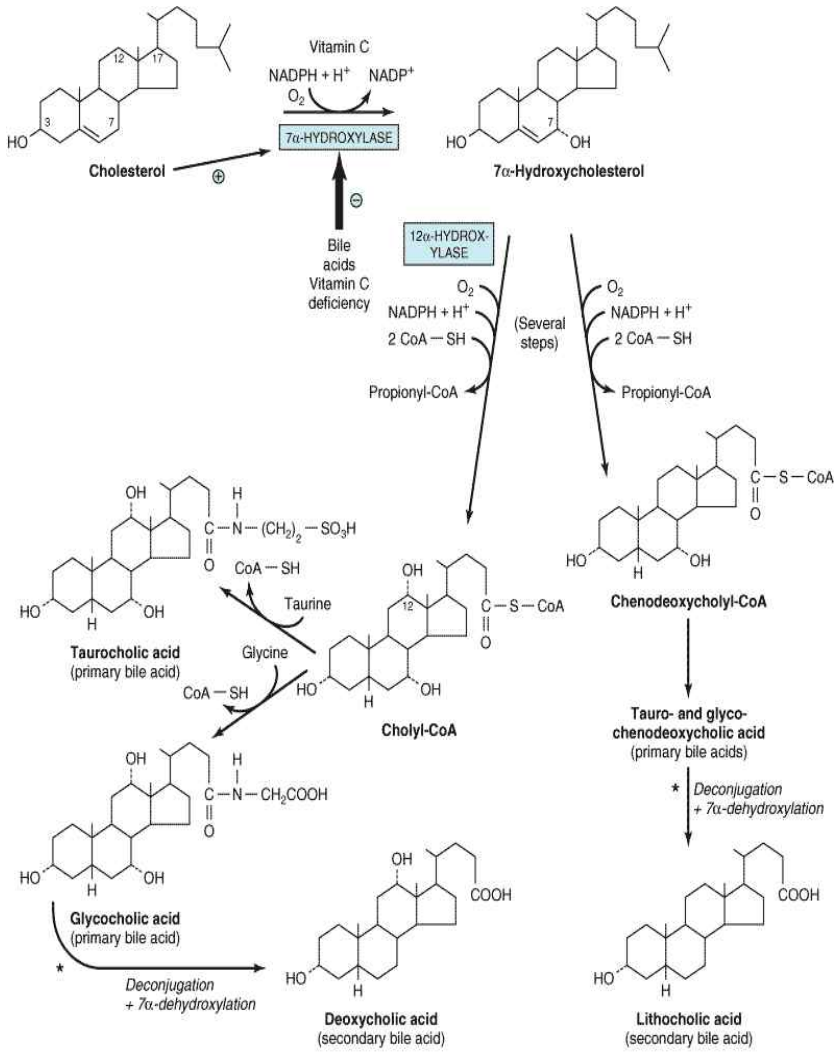

Figure 4. Synthesis of bile salts

Bile acids are natural emulsifiers that has either hydrophilic and hydorphobic surface in one molecule enhance lipase's activity by reduce tension of oil-water interface (Chen et al., 1975). And secreted bild acids are almost absorbed (approximately 95%) in

the ileum via the portal circulation. They are absorbed by the liver and transported to gall bladder for storage and releas back into the duodenum. This process is known as the enterohepatic circulation.

Table 5. Level of bile acids in the bile of the chicken and duck (mg/g)

Bile acid	Chicken	Duck
Cholec acid	9.6 ± 0.5	45.2 ± 2.3
Chenodeoxycholic acid	25.2 ± 2.2	28.2 ± 1.6
Ursodeoxycholic acid	ND	43.5 ± 2.1
Deoxycholic acid	ND	31.6 ± 1.9
Lithocholic acid	68.7 ± 2.1	37.5 ± 2.1
Taurocholic acid	152.6 ± 3.1	16.8 ± 1.5
Taurochenodeoxycholic acid	ND	97.5 ± 3.4
Taurolithocholic acid	35.9 ± 0.6	ND
Glycolithocholic acid	228.4 ± 1.6	ND

ND = not detected.

Source: Yeh and Hwang (2001)

Bile secretion in young birds is very low, it is thought to be limiting factor of fat digestion and absorption (Krogdahl, 1985). And young birds can not replenish bile salts as older birds pool size of bile salts is decreased lead poor fat digestibility (Serafin and Nesheim, 1970). So several studies were conducted to evaluate the effect of bile salts supplementation.

In the early study of Fedde et al. (1960), chicks fed with 0.5% of bile had higher fat absorption than those fed diet without bile. And with increasing levels of bile addition (0, 0.05, 0.1, 0.5, 1, 2, 4 and 8%), fat digestibility was higher in group fed with 0.5% bile than group fed with lower amount of bile. And the digestibility was not increase more with above 0.5% addition level. Gomez and Polin (1976) reported that addition of bile acid (cholic and chenodeoxycholic acids) and bile salts (taurocholate) at 0.025 and 0.05% to the broiler diet that containing 8.2% of tallow improved fat digestibility at 7 and 19 d of age. And Polin et al. (1980) also found that 0.04% of bile acids (cholic acid, chenodeoxycholic acid, deoxycholic acid) or bile salt sodium taurocholate) supplementation increase fat digestibility at 1 and 3 weeks of age broiler that fed diets containing 4% tallow. In this study group fed chenodeoxycholic acid showed higher fat absorption at 1 week of age and at 3 weeks of age cholic acid group had higher fat absorption. Kussaibati et al. (1982) found that supplementation of bile salts increased digestibility of animal-vegetable blend fats that has less saturated fats. And Alzawqari et al. (2011) studied the effect of bile on growth performance and fat digestibility of broilers. Bile was supplemented with three levels (0, 0.25, and 0.5%) to the diets containing tallow (5%) and birds fed with 0.5% of bile had higher weight gain and fat digestibility and lower feed per gain ratio than birds fed with 0, or 0.025% of bile.

2.4 Effects of exogenous emulsifier on lipid digestibility

Lipids are not soluble in water so they exist as big globule in gastrointestinal tract. To make it easy to digested by lipolytic enzymes they need to be emulsified and dispersed resulting in increase surface area. Therefore emulsifiers can improve the utilization of lipids and it's role may especially effective in young birds that has limitation of bile production and recirculation.

2.4.1 Lecithin

Lecithin is phospholipids that usually made form soybean. By the material that incorporate with phosphate group, there are some isomers of phospholipids. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidic acid are some of that kinds of substances and phosphatidylcholine is most common in nature.

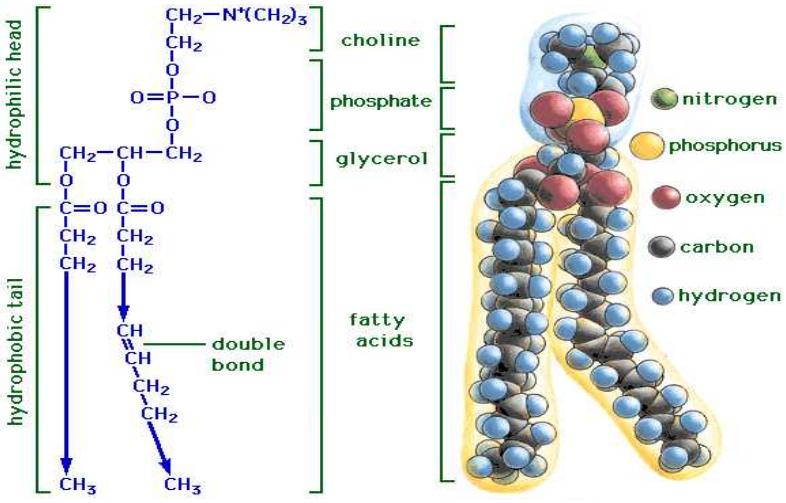

Figure 5. Molecular structure of phosphatidylcholine

These are widely used in food industry as emulsifier and has potential to improve digestibility of dietary fat in animal feed. It improves fat digestibility in diets for calves (Hopkins et al., 1959), young pigs (Overland et al., 1993; Soares and Lopez Bote, 2002) and broilers (Polin, 1980).

Jones et al. (1992) reported tallow was more digestible with 0.05%, 0.1%, and 0.3% of lecithin addition in weaning pigs. Jin et al. (1998) also found that group fed diet that contained 9% of tallow with 1% of lecithin had significantly high growth rate and feed efficiency of weaning pigs and increased digestibility of gross energy, dry matter, fat and crude protein than those fed diet that contained 10% of tallow. In broiler studies, 2% of lecithin supplementation to diet that containing 4% tallow increased fat digestibility compared to those fed 0.02% and 0.2% of lecithin (Polin, 1980). Huang et al. (2007) reported that final body weight of broiler (42d old) and fat digestibility was improved when 0.5% of lecithin added to diet containing 1.5% of soy oil.

Otherwise there's some studies that found no positive effect of dietary lecithin. Overland et al. (1993) reported that addition of lecithin (1, 2, and 3%) to weaning pigs diet did not affect weight gain, feed intake, gain/feed during d 0 to 35 postweaning. Also Blanch et al. 1995 observed addition of 0.2% lecithin did not have positive effect on digestibility of fatty acids and apparent metabolizable energy. In broiler studies, Azman and Ciftci (2004) found no differences in the feed per gain of broilers

that fed diet containing soybean oil with 1% and 2% of lecithin. And in another study, 2.5% and 5% of lecithin addition in broiler diets did not affect final body weight and feed conversion ratio (Cantor et al., 1997).

These disagreement of results among the studies may be contributed by different content of lecithin and formula of diet especially type of fat and their content.

And lysolecithin which is comes from lecithin by enzyme treatment also has same emulsifing property. But as one of fatty acid of lecithin is removed, lysolecithin has more hydrophilic character. Supplementation of lysolecithin has positive effect on growth performance (De Rodas, 1995; Danek et al., 2005), and fat digestibility in weaning pigs (Jones et al. 1992; Xing et al., 2004; Danek et al., 2005). Also lysolecithin addition to broiler diets improved growth performance and nutrients digestibility (Mlelegy et al., 2010; Zhang et al. 2011).

2.4.2 Sodium stearoyl-2-lactylate

Sodium stearoyl-2-lactylate is a commercially available lactylate that is mixture of sodium salts of stearoyl lactylic acids and minor proportions of other sodium salts of related acids. As Food and Drug Administration (FDA) approved food additive, it is widely used as emulsifier. Because it has high hydrophilic property (hyrophilic-lipophilic balance (HLB) is 10-12), it could be apply for feed additive. But it has not been studied a lot. Jeong et al. (2009) reported that supplementation of sodium stearoyl-2-lactylate improved marbling score and meat quality

grade of Hanwoo steers but there was no effect on growth rate, feed efficiency. Choi et al. (2013) also found sodium stearoyl-2-lactylate addition to Hanwoo steers for fattening period has positive effect on meat quality and growth performance. Moon (2012) evaluated the effect of sodium stearoyl-2-lactylate in swin and found sodium stearoyl-2-lactylate addition improve growth performance and nutrient digestibility of weaning pigs. Also Choi (2014) observed growth performance and feed efficiency improvement with sodium stearoyl-2-lactylate addition in broiler.

3. Supplementation of lysophospholipids in broiler diets

3.1 General information of lysophospholipids

Lysophospholipids, also called lysolecithins are result from partial hydrolysis of phospholipids which removes one of the fatty acid groups by enzyme phospholipase A (D'Arrigo and Servi, 2010). Phospholipids are major component of all cell membranes as they have aggregation properties and can form lipid bilayers. Commercial product of phospholipids and lysophospholipids are usually come from soy beans, sunflower and rape seeds, egg yolk. And they are widely used in foods, cosmetics, agrochemicals and pharmaceuticals (Reblova and Pokorny, 1995; Liu and Ma, 2011)

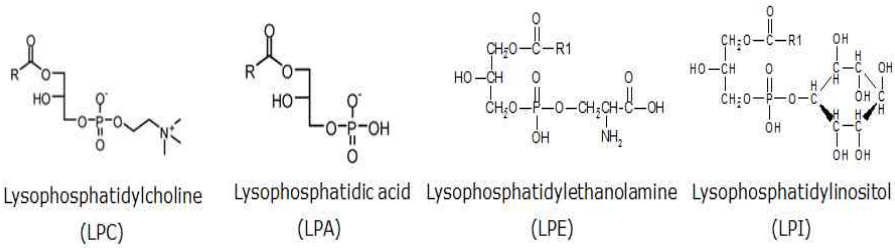

Figure 6. Chemical structure of 4 major lysophospholipids

Emulsifiers are usually classified by HLB value. The HLB value calculated by molecular weight ratio of hydrophilic group per total molecular weight as Griffin's equation as follows:

$$\text{HLB} = 20 \times \frac{\text{Mh (molecular mass of hydrophilic group)}}{\text{M (molecular mass of whole group)}}$$

The closer 20 of HLB value, the easier emulsifier can disperse lipids in aqueous environment. And the other way, low HLB value emulsifiers are useful for water in oil condition. Lysophospholipids' HLB value is about 12, which are higher than value of phospholipids and similar with deoxycholate, known as major component of bile acid. It indicates that lysophospholipids are suitable emulsifier for aqueous environment such as gastrointestinal tract.

Furthermore, it arrange themselves into small particle of micelles and their critical micelle concentration (CMC), that defined as concentration of surfactants above which micelles form, is 0.02-0.2 mM/L that are more effective than bile salts (CMC 4 mM/L) and phospholipids (CMC 0.3-2 mM/L) (Zubay,

1983; Langmuir, 2002). Droplet size is also smaller than phospholipid (Mine et al., 1993) so it may has better capacity for nutrients absorption.

3.2 Function of lysophospholipids on nutrients absorption

Due to their amphipathic property and ability to form micelle, lysophospholipids help in fat digestion. In an intestinal track that aqueous environment, dietary fat could not dispersed and existed as fat globules. Lysophospholipids that have hydrophilic, lipophilic property act as biosurfactants when mixed with water and fat, make fat globules dispersed and make it easy to interact with lipase. And lysophospholipids spontaneously form micelles and liposomes which can fuse into the gut membrane releasing their contents into the blood increasing absorption of both fat soluble nutrients and water soluble nutrients (Melegy et al., 2010). And because of lysophospholipis molecular structure that has only one fatty acid, it compose smaller and stable micelle that can easily across the cell membrane (Reynier et al., 1985). Also it has energy sparing effect. By enhanced fat digestion, it spares the energy for excess bile synthesis.

And lysophospholipids act as absorption enhancer as they can enter into the lipid bilayer of gut membrane, increasing fluidity and permeability of membrane (Shier et al. 1976; Khidir et al., 1995). By increasing membrane's permeability, lysophospholipids may affect mucosal barrier function to

encourage macromolecules such as proteins across the membrane into the blood (Tagesson et al., 1985).

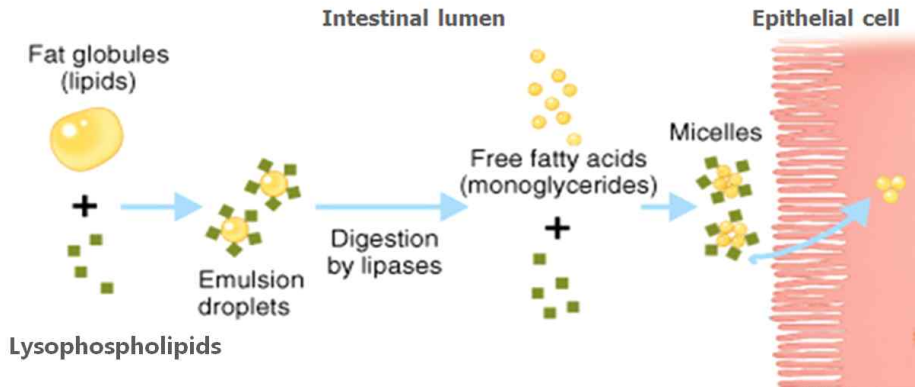

Figure 7. Lysophospholipids in fat digestion and absorption

3.3 Effects of exogenous lysophospholipids in broiler on growth performance and nutrients digestibility

Several studies were conducted to evaluate the effect of lysophospholipids supplementation and found that it enhance broiler's growth performance and nutrient digestibility.

Liu (1997) conducted industrial scale broiler trial (0-47 d) and found addition 0.05% of lysophospholipids improved body weight (BW) and feed conversion ratio (FCR). Melegy et al. (2010) also reported 0.025% and 0.05% supplementation improved BW, body weight gain (BWG), FCR while total feed consumption was not significantly different. Group fed with lysophospholipids had lower mortality and liver weight. Especially, lysophospholipids supplementation could compensate for nutrients

reduction that group fed diet that has low ME (35kcal/kg) and low amino acids (4%) showed same growth performance with control group. Zhang et al. (2011) observed 0.05% of supplementation with lysophospholipids increased BWG in starter period (0-21 d) and tend to improve FCR. But there's no effect in grower period (1-42 d).

Schwarzer and Adams (1996) reported that 0.1% of lysophospholipids treatment increased the ME content of the feed, nitrogen-retention and fat digestibility. 0.05% supplementation also improve apparent digestibility of ME and some fatty acids in strater period of broiler in the study of Zhang et al. (2011). Digestibility of crude protein and dry matter was not affected in that study but Han et al. (2010) who conducted laying hen experiment, found that 0.1% inclusion of lysophospholipids enhance ileal digestibility of crude protein, fat, 10 of essential amino acids.

Ⅲ. Effect of Lysophospholipids Supplementation on Growth Performance, Nutrient Digestibility, Blood Profiles and Carcass Traits in Broilers

Abstract: This study was conducted to evaluate effects of dietary lysophospholipids supplementation to low-energy diet on growth performance, nutrient digestibility, blood profiles, and carcass traits in broilers. A total of 300 male (Ross308®) day-old broilers, with an average initial body weight of 47.0 g were allotted to 1 of 5 dietary treatments with 6 replicates and 10 chicks per pen in randomized complete block (RCB) design. The five dietary treatments were : 1) positive control (PC; T1): the diet was 3,025 (starter period), 3,150 (grower period), and 3,200 kcal/kg (finisher period) of ME that recommended by the breeding company; 2) negative control (NC; T2): the diets contained less 150 kcal/kg of ME than PC treatment; 3) T3: NC + lysophospholipids 0.05%; 4) T4: NC + lysophospholipids 0.10%; 5) T5: NC + lysophospholipids 0.15%. Results revealed that the birds fed with lysophospholipids had greater body weight (BW), body weight gain (BWG) and better feed conversion ratio (FCR) during grower and finisher periods than that of birds in NC ($P<0.01$). The supplementation of lysophospholipids showed positive responses to BW, BWG and FCR during grower, finisher

and overall periods ($P<0.01$). But no significant difference was detected in feed intake (FI) throughout the entire experimental periods ($P>0.05$). Supplementation of lysophospholipids had a positive effect on digestibility of dry matter ($P<0.05$), crude protein ($P<0.01$) and fat ($P=0.08$) compared with NC. Especially dry matter and crude protein digestibility had were improved by lysophospholipids addition ($P<0.01$) whereas digestibility of amino acids had no significant difference among treatments. The relative organ weights of abdominal fat, bursa of fabricius, and spleen of birds were not affected by dietary treatments, however, the relative weight of breast muscle was increased with lysophospholipids inclusion ($P<0.05$). Moreover, at the level of 0.15% lysophospholipids, the relative weight of liver was decreased compared to NC ($P<0.05$). No significant differences were observed in aspartate transaminase, total cholesterol, triglyceride, low density lipoprotein cholesterol and high density lipoprotein cholesterol among treatments. Consequently, dietary lysophospholipids could be used to low energy diet of broilers in order to improve growth performance, feed efficiency, carcass compositions with improvement of nutrients digestibility and without any detrimental effects on lymphoid and metabolic function of organ.

Key words : Lysophospholipids, Low energy diet, Growth performance, Nutrient digestibility, Blood profiles, Carcass traits, Broiler

Introduction

As chicken meat consumption has been increased, broiler breeders have been made chicken grow faster and their nutrient requirements also increased to fulfill their high growth potential. Therefore commercial broilers today are normally fed high energetic ingredients to meet the nutritional requirements. Especially high level of lipids such as animal fats and vegetable oils are included in diets that are well-established as the excellent energy yielding ingredients to improve productivity (Blanch et al., 1996). Conversely, gastrointestinal lumen of newly hatched chicks is too poorly matured to utilize dietary lipid due to lower secreting bile salts and lipolytic enzymes (Noy and Sklan, 1998). These substances play a pivotal role to catalyze lipids into free fatty acids and mono-glycerides, thereafter these degraded materials are transported in the form of mixed micelles (Leeson and Atteh, 1995; Crespo and Esteve-Garcia, 2001). Limitation of any one of these essentials may impair the mechanism of absorption and digestion of lipid. To overcome these problems, some solutions are suggested such as use lipids that has high digestibility, supplement of lipase and/or emulsifier. One of dietary emulsifiers, lysophospholipids are also considered to be an option.

Lysophospholipids are produced from phospholipids that are major component of all cell membranes by enzyme phospholipase

A (D'Arrigo and Servi, 2010). Because of it's structure that has hydrophilic head and lipophilic tail, it has amphiphatic property that could disperse lipid globule into the aqueous environment like gastrointestinal tract. Furthermore, it composes of small particle of micelles that has a critical micelle concentration (CMC) of 0.02-0.2 mM and droplet size approximately 2.4 μm (Zubay, 1983; Langmuir, 2002) therefore its activity is more effective than bile salts and phospholipids (Mine et al., 1993). It has an ability to arrange themselves into mixed micelles which can fuse into the gut membrane releasing their contents into the blood, increasing absorption of both fat soluble and water soluble nutrients (Melegy et al., 2010). Some publications observed some beneficial effects of emulsifiers in swine and poultry diets. For instance, emulsifier improved feed conversion ratio and growth performance of broiler chickens (Guerreiro Neto et al., 2011), including carcass traits, high density lipoprotein (Huang et al., 2008), and decreased amount of free fatty acids and cholesterol in blood (Jones et al., 1992). However, the results of carcass compositions and cholesterol (also their fractions) are inconsistent (Azman and Ciftci, 2004; Roy et al., 2010). Recently, Han et al. (2010) reported that 0.1% lysophospholipids supplementation enhanced fat and amino acids digestibility. However, scant publications are available on low energy diet with various levels of lysophospholipids in broiler. Consequently, the aim of the present study was to evaluate effects of dietary lysophospholipids supplementation to low energy diet on growth performance,

nutrient digestibility, blood profiles, and carcass traits in broiler.

Materials and Methods

Bird Husbandry

A total of 300 male one-day-old Ross 308® broiler chicks, with an average body weight of 47.00 ± 0.00 g was obtained from a local hatchery and vaccinated against Newcastle and Infectious bursa diseases before arrive to Seoul National University experimental farm located in Suwon, Kyunggi-do, South Korea. Chicks were allotted to 1 of 5 dietary treatments with 6 replicates and 10 chicks per pen in randomized complete block (RCB) design. And ambient temperature of house was maintained at 30 ℃ for first 3 days, then substantially decreased 3 ℃ weekly until 20 ℃. The lighting regimen was controlled at 23 hours light : 1 hour dark during the first 7 days of age and 20 hours light : 4 hours dark for the rest experimental period. Fresh water and feed were provided *ad libitum* to all experimental chicks with pan feeders and bell-drinkers.

Experimental Design and Diet

The five dietary treatments were ; 1) positive control (PC; T1): the diet was 3,025 (starter period), 3,150 (grower period), and 3,200 kcal/kg (finisher period) of ME that recommended by the breeding company; 2) negative control (NC; T2): the diets contained less 150 kcal/kg of metabolizable energy (ME) than PC treatment; 3) T3: NC + lysophospholipids 0.05%; 4) T4: NC + lysophospholipids 0.10%; 5) T5: NC + lysophospholipids 0.15%.

The experimental diets were formulated to meet or exceed the nutritional recommendations of Ross 308 Nutrition Specification 2007 and consisted of specific feeding program for starter (d 1-7), grower (d 8-21) and finisher periods (d 22-35: Tables 1, 2 and 3, respectively). The lysophospholipids were obtained from Pathway Intermediates International, Inc. (South Korea) under the commercial trade mark Lipidol®. The lysophospholipids were prepared from phospholipids present in soybean lecithin by phospholipase enzymes. One of fatty acid chain of phospholipids was removed by enzymes thereby encourages interaction with cell membrane and hydrophilic property thus accelerating nutrients absorption

Growth Performance

Chicks were weighed individually for check initial body weight at the start of experiment. And each end of feeding phase, chicks and residual feed per replicate were weighed to calculate body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). The percentage of mortality was recorded daily.

Blood Collection and Serum Analyses

On d 35, six birds with an average body weight form each treatment was selected for anatomy trial. Blood sample were collected from a jugular vein of 30 broilers. The blood samples (approximately 5 mL) were immediately transferred into serum

tube (BD Vacutainer), thereafter the whole blood were centrifuged at 1,500g for 15 min at 4 ℃, and then sera were carefully transferred to 1.5 ml micro-tubes and stored at -20 ℃ until further analysis. Sera of chickens were analyzed concentration of total cholesterol (TC), triglyceride, low density lipoprotein cholesterol (LDL cholesterol), high density lipoprotein cholesterol (HDL cholesterol), and aspartate transaminase (AST) by blood analyzer (Modular analytics, PE, Roche, Germany) with enzymatic colorimetric assay methods for TC, LDL cholesterol, HDL cholesterol and IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) method for AST.

Carcass Traits

After collecting a whole blood, the birds were sacrificed and then internal organs (liver, spleen, bursa of Fabricious and abdominal fat), breast and leg muscles were separated and weighted immediately. The relative weight of those organs and muscles were calculated.

Nutrient Digestibility

A metabolic trial was conducted from 17-23 d of age. Thirty chicks were individually placed and raised in a metabolic cage for 7 days with 4 days of adaptation and 3 days of fecal collection. Finisher diet was provided daily with free access to water and mixed 0.5% ferric oxide as an indigestible marker at the final day of the experiment. During 21 to 23 d of age, fecal

samples (without feathers and scales) were collected from each replicate, dried in an air-force drying oven at 60 ℃ for 72 h, ground into 1 mm particles in a Wiley mill, and stored in sealed plastic bags at 4 ℃ until analysis.

Coefficients of apparent digestibility of dietary nutrients were calculated as following equation:

$$\text{X apparent digestibility} = \frac{\text{X ingested} - \text{X excreted}}{\text{X ingested}} \times 100,$$

where X represents dry matter (DM), crude protein (CP), ether extract (EE) and amino acid (AA).

Chemical Analysis

Diets and dried-excreta were analyzed for DM (procedure 967.03; AOAC, 1995) and EE (Folch et al., 1957). The content of CP in excreta was calculated as following equation: CP = total nitrogen x 6.25 (procedure 981.10, AOAC, 1995).

Total 17 amino acids(lysine, methionine, cysteine, threonine, valine, leucine, isoleucine, arginine, histidine, phenylalanine, alanine, aspartic acid, glutamic acid, glycine, proline, tyrosine, and serine) were analyzed. To determine amino acids contents of the diets and digesta, approximately 100 mg of samples were hydrolyzed with 6 N HCl for 24 h at 110 ℃ to allow the release of amino acids from protein molecules (Mason, 1984) and then analyzed by amino acids analyzer (L-8900; Hitachi High-Technologies Corp., Tokyo, Japan) with Ninhydrin method.

Statistical Analysis

Data were analyzed by one-way ANOVA using general linear models of Statistical Analysis System program (SAS institution, 2004) in a randomized complete block design. Significant differences among each group were determined using least significant difference (LSD). Linear and quadratic effects for equally spaced treatments were assessed by orthogonal polynomial contrast to determine the effect of supplementation level of dietary lysophospholipids on all measurements. Significant differences were declared at $P<0.05$ or highly significant at $P<0.01$ and the determination of tendency for all analysis was $P>0.05$ and $P<0.10$.

Results and Discussion

Growth performance

Results for body weight, body weight gain, feed intake and feed conversion ratio during starter (1-7 d), grower (8-21 d), finisher (22-35 d) periods were represented in Table 4. Mortality was not observed in whole experimental period (data not provided). There were no significant differences in BW, BWG, FI and FCR at 7 d of age. These results were consistent with the findings of Guerreiro Neto et al. (2011) and this is possibly because of poor digestive enzyme activity during this period (Nir et al., 1993). By contrast, during grower and finisher periods the birds fed with lysophospholipids had greater BW, BWG and better FCR than that of NC ($P<0.01$). And group fed diet with lysophospholipids showed no significantly difference in BW, BWG during grower, finisher and overall periods with PC group ($P<0.01$). In case of FCR, it improved as lysophospholipids addition level increased and 0.15% treatment group showed no significantly difference with PC group ($P<0.01$). These results were in agreement with the results of Melegy et al. (2010) who observed 42 day old broilers that fed on lower nutrient density diets (lower in oil and synthetic amino acids) with lysophospholipids had higher BW, BWG, and better FCR than that of fed same diets without lysophospholipids. And there was no significant difference in FI among the treatments throughout the whole experimental period.

It can be explained that the lysophospholipids has positive effects on the emulsifying capacity, activity to form mixed micelles and liposomes, modification of gut membrane that could enhance absorption of lipid and other nutrients in digesta (Melegy et al., 2010; Zhang et al., 2011; Guerreiro Neto et al., 2011; Aguilar et al., 2013). These specific properties modulate the incorporation of nutrients into mixed micelles and liposomes in the intestinal lumen and release their components into the bloodstream, result in getting more weight gain and improving feed conversion ratio (Melegy et al., 2010). Also Jones et al. (1992) and Guerreiro Neto et al. (2011) revealed that the emulsified fat particles increasing pancreatic lipase secretion.

Nutrient Digestibility

The results of nutrient digestibility were indicated in Table 5. The birds fed diet with lysophospholipids had higher digestibility of dry matter than those fed with NC ($P<0.05$) and showed linear response by inclusion rate ($P<0.01$). Crude protein digestibility were also improved than that of NC ($P<0.01$) in the linear and quadratic response ($P<0.01$). Lysophospholipids addition improved digestibility of DM, CP and showed no significantly difference with digestibility in PC treatment ($P<0.01$). Moreover, lysophospholipids treatment tended to increase ($P=0.08$) the fat digestibility. However, this research was not found significant effects on apparent digestibility of amino acids. These findings were consistent with Zhang et al. (2011) reported supplementation

with lysophospholipids to broiler increased digestibility of apparent ME and some fatty acids during the starter (14-17 d) and grower (35-38 d) period. Also Han et al. (2010) observed improvement of crude protein and fat ileal digestibility when lysophospholipids were supplemented to laying hen.

In this study, it was observed that providing 0.10% of lysophospholipids to low energy diet of broiler diet seemed to be selecting and consuming more of the energy portion of the diet on a daily basis. Subsequently, they may have been getting more energy from the diet than chicks in the NC treatment even they had same feed intake in other words had same energy intake. The advantageous effects of the lysophospholipids on growth performance and feed efficiency of broilers may be associated with bioavailability of nutrients, particularly in that of crude protein and fat, which increased significantly when the lysophospholipids was supplemented to diet. Because of lysophospholipids' structure, micelles can be formed more smaller and stable than those formed with phospholipids (Zhang et al., 2011). The micelles size is an important factor that determined the absorption of nutrients, formation of smaller micelles may also have an effective response on digestion and absorption of both fat soluble nutrients and water soluble nutrients (Roy et al., 2010). Another feature of lysophospholipids is that individual of this can enter into the gut membrane leading to increase the porosity of the membrane and nutrients absorption across the membrane (Melegy et al., 2010).

From the finding, providing lysophospholipids can improve the digestibility of dry matter, crude protein and fat so that it may compensate for the low energy content of diet.

Carcass Traits

The supplemenatation of lysophospholipids did not affect the relative weight of abdominal fat pad, bursa of Fabricius and spleen during 35-d feeding period in broiler chickens (Table 6). However, dietary treatments fed with lysophospholipids at the levels of 0.05 and 0.10% increased the relative organ weight of the breast muscle compared to NC group ($P<0.05$). Additionally, when birds were fed the highest level of lysophospholipids (0.15%) the relative organ weight of leg muscle was greater than that of birds in the 0.05% lysophospholipids treatment ($P<0.01$). A significant effect was also showed a lower relative weight on liver size compared to NC ($P<0.05$). This result was coincident with the previous study of Melegy et al. (2010) who found liver index was significantly lower and there was no effect on spleen and bursa index with lysophospholipids treatment. Although there was no effect on breast and leg muscle, Guerreiro Neto et al. (2011) also reported there was no influence of emulsifier on carcass traits (breast muscle, leg muscle, abdominal fat) while breast muscle was increased by lysophospholipids treatment. The lysophospholipids have an ability to enhance utilization of nutrients including lipid, it may facilitate their conversion into the breast and leg muscles.

By contrast, the enlargement of the liver was observed in NC. In birds, lipid anabolism takes place primarily in the liver (Leveille et al., 1975) so lower energy diet can activate the metabolic pathway in the liver to catalyze macromolecules in order to provide energy sources for maintenance and peripheral tissues accumulation (Krogdhal, 1985).

In the current trial, exogenous lysophospholipids are able to be supplemented in low-energy diet of birds without any detrimental effect on meat quality of broiler chickens. Furthermore, birds showed a normal histology in the spleen and bursa of Fabricius weights that indicated there was no immunologically harmful effect.

Blood Profiles

Serum concentration of aspartate transaminase, triglyceride, cholesterol and their fractions in broiler chicks were presented in Table 7. There were no significant differences among all treatments. These results were coincident with Melegy et al. (2010) that aspartate transaminase, triglyceride and cholesterol were not affected by lysophospholipids. Guerreiro Neto et al. (2011) demonstrated that triglyceride, high density lipoprotein and cholesterol were not affected by emulsifier.

Aspartate transaminase, the hepatic enzyme activity, is generally used as an indicator of liver damage in both human and animals, and lower level is considered to be more favorable (Parke and Ioannides, 1981). From this study, the

lysophospholipids supplementation in broilers diets had no negative effect on liver despite of high availability of dietary DM, CP and fat. And this might be explained by the effect of phospholipids on liver that improving the liver function (Attia et al., 2009). The concentration of cholesterol, triglyceride and lipoprotein was not affacted by dietary treatments. This result demonstrated that fat metabolism were not adversely affected by lysophospholips supplementation. As blood profile can reveals subclinical disorders resulting from malnutrition, these results indicated that there were no alterations in liver and fat metabolism with lysophospholipids supplementation.

Conclusion

Dietary lysophospholipids can significantly improve broiler performance during grower and finisher periods by enhancing nutrient absorption without negative effects on carcass trait and metabolic status. In growth performance, there was no significant difference in feed consumption among treatments during the entire experimental period. Although FCR was not affected by dietary treatments during a starter period (1-7 d of age), a linear response ($P<0.05$) was shown in groups fed with low-energy diets with lysophospholipids in grower and finisher periods. Consequently, dietary lysophospholipids could be supplemented to low energy diet (decrease 150 kcal/kg of ME from a standard energy requirement) at 0.15% levels in order to improve growth performance, feed efficiency, nutrient digestibility, muscle accumulation without growth retardation, hepatic enlargement and metabolic homeostasis.

Table 1. Composition of experimental diets (Phase I, from 0 to 7 d)

Ingredients (%)	Positive control (PC)	Negative control (NC)	NC + Lysophospholipids(%)		
			0.05	0.10	0.15
Corn	54.85	55.99	55.94	55.89	55.84
Wheat bran	1.00	2.97	2.97	2.97	2.97
Soybean meal	34.99	34.18	34.18	34.18	34.18
Fish meal	1.00	1.00	1.00	1.00	1.00
Soybean oil	3.32	1.00	1.00	1.00	1.00
L-Lysine, 55%	0.415	0.433	0.433	0.433	0.433
DL-Methionine, 98%	0.392	0.389	0.389	0.389	0.389
Threonine, 98%	0.117	0.120	0.120	0.120	0.120
Choline chloride, 50%	0.06	0.06	0.06	0.06	0.06
Dicalcium phosphate	2.12	2.14	2.14	2.14	2.14
Limestone	1.10	1.09	1.09	1.09	1.09
Salt	0.33	0.33	0.33	0.33	0.33
$NaHCO_3$	0.06	0.06	0.06	0.06	0.06
Vitamin premix[1]	0.125	0.125	0.125	0.125	0.125
Mineral premix[2]	0.12	0.12	0.12	0.12	0.12
Lysophospholipids[3]	0.00	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition[4]					
Moisture (%)	12.20	12.51	12.51	12.51	12.51
Crude protein (%)	22.00	22.00	22.00	22.00	22.00
Ether extract (%)	5.88	3.67	3.67	3.67	3.67
Fiber (%)	2.27	2.46	2.46	2.46	2.46
Ash (%)	6.00	6.06	6.06	6.06	6.06
Calcium (%)	1.05	1.05	1.05	1.05	1.05
Phosphorus (%)	0.78	0.79	0.79	0.79	0.79
ME (kcal/kg)	3,025	2,875	2,875	2,875	2,875
Lysine (%)	1.43	1.43	1.43	1.43	1.43
Methionine+Cystine (%)	1.07	1.07	1.07	1.07	1.07
Threonine (%)	0.94	0.94	0.94	0.94	0.94
Tryptophan (%)	0.26	0.26	0.26	0.26	0.26

[1] Provided the following quantities of vitamins per kg of complete diet: vitamin A 11,000 IU; vitamin D_3 5,000 IU; vitamin E 60 mg; vitamin K 3 mg; vitamin B_1 3 mg; vitamin B_2 8 mg; vitamin B_6 4 mg; vitamin B_{12} 16 μg; niacin 60 mg; folic acid 2 mg; Biotin 130 μg; calcium pantothenic acids 20 mg.

[2] Provided the following quantities of minerals per kg of complete diet: Cu 29 mg; Zn 108 mg; I 1 mg; Mn 115 mg; Fe 60 mg; Se 0.4 mg.

[3] Lipidol® (Pathway Intermediates International, Inc)

[4] Calculated values. as-fed basis.

Table 2. Composition of experimental diets (Phase II, from 8 to 21 d)

Ingredients (%)	Positive control (PC)	Negative control (NC)	NC + Lysophospholipids(%)		
			0.05	0.10	0.15
Corn	55.65	57.14	57.09	57.04	56.99
Wheat bran	1.00	2.68	2.68	2.68	2.68
Soybean meal	33.35	32.57	32.57	32.57	32.57
Fish meal	1.00	1.00	1.00	1.00	1.00
Soybean oil	5.11	2.70	2.70	2.70	2.70
L-Lysine, 55%	0.154	0.171	0.171	0.171	0.171
DL-Methionine, 98%	0.290	0.287	0.287	0.287	0.287
Threonine, 98%	0.036	0.039	0.039	0.039	0.039
Choline chloride, 50%	0.05	0.05	0.05	0.05	0.05
Dicalcium phosphate	1.86	1.87	1.87	1.87	1.87
Limestone	0.87	0.86	0.86	0.86	0.86
Salt	0.33	0.33	0.33	0.33	0.33
$NaHCO_3$	0.06	0.06	0.06	0.06	0.06
Vitamin premix[1]	0.125	0.125	0.125	0.125	0.125
Mineral premix[2]	0.12	0.12	0.12	0.12	0.12
Lysophospholipids[3]	0.00	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition[4]					
Moisture (%)	12.07	12.39	12.39	12.39	12.39
Crude protein (%)	21.00	21.00	21.00	21.00	21.00
Ether extract (%)	7.66	5.36	5.36	5.36	5.36
Fiber (%)	2.24	2.42	2.42	2.42	2.42
Ash (%)	5.46	5.51	5.51	5.51	5.51
Calcium (%)	0.90	0.90	0.90	0.90	0.90
Phosphorus (%)	0.73	0.74	0.74	0.74	0.74
ME (kcal/kg)	3,150	3,000	3,000	3,000	3,000
Lysine (%)	1.24	1.24	1.24	1.24	1.24
Methionine+Cystine (%)	0.95	0.95	0.95	0.95	0.95
Threonine (%)	0.83	0.83	0.83	0.83	0.83
Tryptophan (%)	0.25	0.25	0.25	0.25	0.25

[1] Provided the following quantities of vitamins per kg of complete diet: vitamin A 11,000 IU; vitamin D_3 5,000 IU; vitamin E 60 mg; vitamin K 3 mg; vitamin B_1 3 mg; vitamin B_2 8 mg; vitamin B_6 4 mg; vitamin B_{12} 16 μg; niacin 60 mg; folic acid 2 mg; Biotin 130 μg; calcium pantothenic acids 20 mg.

[2] Provided the following quantities of minerals per kg of complete diet: Cu 29 mg; Zn 108 mg; I 1 mg; Mn 115 mg; Fe 60 mg; Se 0.4 mg.

[3] Lipidol® (Pathway Intermediates International, Inc)

[4] Calculated values. as-fed basis.

Table 3. Composition of experimental diets (Phase III, from 22 to 35 d)

Ingredients (%)	Positive control (PC)	Negative control (NC)	NC + Lysophospholipids(%)		
			0.05	0.10	0.15
Corn	60.6	61.97	61.92	61.87	61.82
Wheat bran	1.00	2.78	2.78	2.78	2.78
Soybean meal	28.47	27.68	27.68	27.68	27.68
Fish meal	1.00	1.00	1.00	1.00	1.00
Soybean oil	5.28	2.90	2.90	2.90	2.90
L-Lysine, 55%	0.121	0.138	0.138	0.138	0.138
DL-Methionine, 98%	0.250	0.247	0.247	0.247	0.247
Threonine, 98%	0.024	0.028	0.028	0.028	0.028
Choline chloride, 50%	0.05	0.05	0.05	0.05	0.05
Dicalcium phosphate	1.72	1.73	1.73	1.73	1.73
Limestone	0.85	0.84	0.84	0.84	0.84
Salt	0.33	0.33	0.33	0.33	0.33
$NaHCO_3$	0.06	0.06	0.06	0.06	0.06
Vitamin premix[1]	0.125	0.125	0.125	0.125	0.125
Mineral premix[2]	0.12	0.12	0.12	0.12	0.12
Lysophospholipids[3]	0.00	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition[4]					
Moisture (%)	12.11	12.43	12.43	12.43	12.43
Crude protein (%)	19.00	19.00	19.00	19.00	19.00
Ether extract (%)	7.92	5.65	5.65	5.65	5.65
Fiber (%)	2.22	2.40	2.40	2.40	2.40
Ash (%)	5.09	5.15	5.15	5.15	5.15
Calcium (%)	0.85	0.85	0.85	0.85	0.85
Phosphorus (%)	0.68	0.69	0.69	0.69	0.69
ME (kcal/kg)	3,200	3,050	3,050	3,050	3,050
Lysine (%)	1.09	1.09	1.09	1.09	1.09
Methionine+Cystine (%)	0.86	0.86	0.86	0.86	0.86
Threonine (%)	0.74	0.74	0.74	0.74	0.74
Tryptophan (%)	0.22	0.22	0.22	0.22	0.22

[1] Provided the following quantities of vitamins per kg of complete diet: vitamin A 11,000 IU; vitamin D_3 5,000 IU; vitamin E 60 mg; vitamin K 3 mg; vitamin B_1 3 mg; vitamin B_2 8 mg; vitamin B_6 4 mg; vitamin B_{12} 16 μg; niacin 60 mg; folic acid 2 mg; Biotin 130 μg; calcium pantothenic acids 20 mg.

[2] Provided the following quantities of minerals per kg of complete diet: Cu 29 mg; Zn 108 mg; I 1 mg; Mn 115 mg; Fe 60 mg; Se 0.4 mg.

[3] Lipidol® (Pathway Intermediates International, Inc)

[4] Calculated values. as-fed basis.

Table 4. Effect of lysophospholipids supplementation to low energy diets on growth performance[1,2]

Items	Positive control (PC)	Negative control (NC)	NC + Lysophospholipids(%)			SEM[3]	*P-value*	
			0.05	0.10	0.15		Linear	Quadratic
Body Weight, g/bird								
Initial	47.00	47.00	47.00	47.00	47.00			
1 week	155.88	154.73	157.42	157.72	158.64	1.126	0.285	0.469
3 week	977.12^{A}	845.60^{B}	954.25^{A}	950.95^{A}	978.69^{A}	11.772	0.001	0.001
5 week	2,078.33^{A}	1,789.00^{B}	1,981.67^{A}	2,024.00^{A}	2,063.67^{A}	22.312	0.001	0.003
Body weight gain, g/bird								
0-1 week	108.88	107.73	110.42	110.72	111.64	1.126	0.285	0.469
1-3 week	821.23^{A}	690.87^{B}	796.83^{A}	793.23^{A}	820.05^{A}	11.591	0.001	0.001
3-5 week	1,101.22^{A}	943.40^{B}	1,027.42^{AB}	1,073.05^{A}	1,084.98^{A}	14.433	0.003	0.050
Overall	2,031.33^{A}	1,742.00^{B}	1,934.67^{A}	1,977.00^{A}	2,016.67^{A}	22.312	0.001	0.003
Feed intake, g/bird								
0-1 week	123	122	125	122	116	1.7	0.253	0.776
1-3 week	1,241	1,207	1,205	1,255	1,225	10.0	0.372	0.525
3-5 week	1,754	1,757	1,794	1,799	1,809	12.2	0.147	0.335
Overall	3,118	3,087	3,124	3,176	3,150	16.9	0.166	0.875
Feed conversion ratio, feed/gain								
0-1 week	1.13	1.14	1.14	1.10	1.04	0.015	0.044	0.426
1-3 week	1.52^{B}	1.75^{A}	1.51^{B}	1.58^{B}	1.49^{B}	0.023	0.002	0.002
3-5 week	1.59^{C}	1.86^{A}	1.75^{B}	1.68^{BC}	1.67^{BC}	0.021	0.002	0.051
Overall	1.54^{C}	1.77^{A}	1.62^{B}	1.61^{B}	1.57^{BC}	0.017	0.001	0.001

[1] A total of 300 broilers were raised for 35 days feeding period.
[2] Least squares means for six pens/treatment with ten broilers/pen.
[3] Standard error of mean.
[A,B,C] Means in a same row with different superscripts were significantly different ($P<0.01$).

Table 5. Effect of dietary lysophospholipids to low energy diets on nutrient digestibility[1,2]

Items (%)	Positive control (PC)	Negative control (NC)	NC + Lysophospholipids(%)			SEM[3]	*P-value*	
			0.05	0.10	0.15		Linear	Quadratic
Dry matter	72.30[a]	69.20[b]	71.19[a]	72.06[a]	71.97[a]	0.335	0.002	0.078
Crude protein	65.88[BC]	64.44[C]	67.72[AB]	69.69[A]	68.00[AB]	0.536	0.001	0.008
Ether extract	88.50	81.25	86.20	85.71	83.36	0.855	0.486	0.065
Amino acids[4]	87.95	86.26	86.40	88.05	86.69	0.311	0.960	0.493

[1] A total of 30 broilers were raised 17 to 23 day-old of age and the initial average body weight was 874.52±23.10 g.
[2] Values are means for six broilers per treatment.
[3] Standard error of mean.
[4] Total 17 amino acids
[a,b] Means in a same row with different superscripts were significantly different (P<0.05).
[A,B] Means in a same row with different superscripts were significantly different (P<0.01).

Table 6. Effect of lysophospholipids on the relative organ weights of chickens[1,2]

Items (g/100 g BW)	Positive control (PC)	Negative control (NC)	NC + Lysophospholipids(%)			SEM[3]	*P-value*	
			0.05	0.10	0.15		Linear	Quadratic
Breast muscle	17.80[a]	16.06[b]	17.86[a]	18.04[a]	17.58[ab]	0.139	0.106	0.298
Leg muscle	17.66[AB]	18.62[A]	16.80[B]	17.90[AB]	18.42[A]	0.099	0.785	0.115
Liver	2.04[ab]	2.12[a]	1.98[ab]	2.01[ab]	1.92[b]	0.030	0.078	0.113
Abdominal fat	2.18	1.88	2.04	1.84	1.72	0.073	0.456	0.901
Bursa of Fabricius	0.25	0.30	0.28	0.24	0.27	0.010	0.138	0.944
Spleen	0.10	0.10	0.11	0.09	0.09	0.003	0.130	0.915

[1] A total of 30 broilers were raised at 35 day-old of age and the average body weight was 1,913.56±18.93 g.
[2] Least squares means for six broilers per treatment.
[3] Standard error of mean.
[a,b] Means in a same row with different superscripts were significantly different ($P<0.05$).
[A,B] Means in a same row with different superscripts were significantly different ($P<0.01$).

Table 7. Effect of lysophospholipids supplementation to low energy diets on blood profiles in broilers[1,2]

Items	Positive control (PC)	Negative control (NC)	NC + Lysophospholipids(%)			SEM[3]	*P-value*	
			0.05	0.10	0.15		Linear	Quadratic
AST, U/L	252.17	283.17	246.17	215.83	260.00	9.648	0.309	0.075
Cholesterol, mg/dL	115.33	119.33	119.67	126.00	119.83	1.640	0.555	0.280
Triglyceride, mg/dL	29.67	28.50	28.50	29.50	31.17	0.841	0.308	0.668
LDL cholesterol, mg/dL	16.00	15.67	18.00	19.50	16.33	0.864	0.700	0.188
HDL cholesterol, mg/dL	92.17	100.67	99.33	102.83	100.50	1.444	0.805	0.854

[1] A total of 30 broilers were raised at 35 day-old of age and the average body weight was 1,987.33±22.31 g.
[2] Values are means for six broilers per treatment.
[3] Standard error of mean.

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Ⅴ. Summary in Korean

본 연구는 저에너지 육계 사료에 lysophospholipids 의 첨가가 성장성적, 영양소 소화율, 혈액성상, 도체성적에 미치는 영향을 검증하고자 수행되었다. 평균 체중이 47.0 g 인 총 300수의 1일령 수평아리 (Ross 308®)를 5개 처리구에 6반복, 반복 당 10수씩 난괴법 (randomized complete block; RCB)으로 배치하였다. 5개 처리구는 1) positive control (PC; T1): 육종회사에서 권장하는 대사에너지가인 3,025 (초기), 3,150 (전기), 3,200 kcal/kg (후기)를 충족시키는 사료; 2) negative control (NC; T2): PC 처리구의 대사에너지가보다 150kcal/kg 낮은 에너지의 사료; 3) T3: NC 처리구 사료에 lysophospholipids를 0.05% 첨가; 4) T4: NC 처리구 사료에 lysophospholipids를 0.10% 첨가; 5) T5: NC 처리구 사료에 lysophospholipids를 0.15% 첨가; 이다. 사양실험 결과 전기와 후기 구간에서 NC 처리구 대비 lysophospholipids를 급이한 처리구들에서 체중과 증체량, 사료요구율이 개선되는 결과를 보였으며 ($P<0.01$), 이 때 첨가수준이 증가할수록 체중과 증체량, 사료요구율이 유의적으로 개선되었다 (linear, $P<0.01$). 하지만 실험 전체 기간 동안 사료섭취량에는 처리구간 차이가 발견되지 않았다 ($P>0.05$). 영양소 소화율에서는 lysophospholipids를 첨가함으로써 건물 ($P<0.05$), 조단백질 ($P<0.01$) 그리고 지방 ($P=0.08$)의 소화율이 NC 처리구 대비하여 개선되었다. 특히 첨가량 수준이 증가함에 따라 건물과 조단백질의 소화율이 유의적으로 증가하는 결과를 보였다 (linear, $P<0.01$). 반면 아미노산의 소화율에서는 처리구간 유의적인 차이가 발견되지 않았다. 복강지방, F낭, 비장의 체중당 무게는 처리구간 차이가 없었

으나 가슴육 무게는 lysophospholipids 첨가에 따라 증가하였다 ($P<0.05$). 또한 간의 경우 lysophospholipids를 0.15% 첨가한 처리구에서 NC 처리구와 비교하여 무게가 감소하는 결과를 보였다 ($P<0.05$). 혈액성상 분석결과 aspartate transaminase, 총콜레스테롤, 중성지방, low density lipoprotein cholesterol, high density lipoprotein cholesterol 함량의 처리구간 차이는 별견되지 않았다. 결론적으로 저에너지 육계사료에 lysophospholipids를 첨가하면 면역이나 대사와 관련된 장기 기능에 부정적인 영향을 주지 않으면서 영양소 이용율을 높이고 성장성적, 사료효율, 도체성상을 개선할 수 있을 것으로 판단된다.