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for the Degree of Doctor of Philosophy

Evaluation of Dietary Lysophospholipids for Broilers Chickens and Laying Hens

사료 내 Lysophospholipids 의첨가가 육계와 산란계에 미치는 영향

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Overall Summary

Evaluation of Dietary Lysophospholipids for Broilers Chickens and Laying Hens

The studies aimed to increase nutrient utilization of broilers and laying hens by the use of lysophospholipid (LPL). This approach would be validated for poultry producers' to minimize feed cost without retarding growth performance and laying productivity. Consequently, three experiments were conducted.

Experimental I. Effects of Lysophospholipid Supplementation to Lower Nutrient Diets on Growth Performance, Intestinal Morphology, and Blood Metabolites in Broiler Chickens

The purpose of this research was to investigate the effects of dietary lysophospholipid (LPL) supplementation to diets lower in metabolizable energy (ME), crude protein including selected amino acids on growth performance, intestinal morphology, blood metabolites, inflammatory response, and carcass traits in broiler chickens. A total of 300 one-day-old male chicks (Ross 308) were assigned to 5 treatments, with 6 replications of 10 birds each in a completely randomized design. The 5 treatments were: positive control (PC) without LPL

supplementation and adequate in all nutrients, negative control (NC) without LPL, and reduced 150 kcal/kg of ME and reduced 5% of total crude protein including Lys, Met, Thr, and Trp in a calculated amount relative to the PC, NC + 0.05% LPL (LPL05), NC + 0.10% LPL (LPL10), and NC + 0.15% LPL (LPL15). Broilers fed with NC diet had poorer growth performance and lighter relative weight of breast muscle compared with PC diet. Moreover, the NC birds were more susceptible to inflammation via modulating the secretions of tumor necrosis factor alpha (P = 0.011), interleukin-1 (P = 0.036) and increasing crypt depth of the jejunum and duodenum. However, feeding LPL linearly improved growth performance, feed conversion ratio, ether extract, and crude protein retention. The LPL supplementation on low-energy and nitrogenous diets showed significant lowering uric acid (P = 0.001) concentration. Furthermore, the inclusion of LPL to the NC diet could alleviate inflammation with tended to decrease crypt depth of the duodenum (P = 0.074) and decreased tumor necrosis factor alpha (P = 0.082). These improvements also influenced carcass composition, especially in relative weights of pancreas breast and leg muscle. Conversely, the LPL supplementation showed no significant effects on relative weights of immune organs, gizzard, and abdominal fat. Overall, LPL promotes growth performance, nutrient utilization, gut health, anti-inflammation, and muscle yields when applied to diets of broiler chickens with lower levels of energy, crude protein including selected amino acids.

Experimental II. Effects of Dietary Lysophospholipid Supplementation on Egg Production, Lipid Metabolism, Yolk Fatty Acid Deposition in Brown Egg-Laying Hens

This study examined the effects of lysophospholipid (LPL) supplementation on egg production, egg quality, lipid metabolism, and yolk fatty acid deposition of brown egg-laying hens from 28 to 38 wks of age. A total of 420 Hy-Line W36 laying hens were allotted into five dietary treatments with six replicates and 14 hens in each treatment based on a completely randomized design. Hens were fed 0 (CON), 0.025 (LPL25), 0.05 (LPL50), 0.075 (LPL75), and 0.10% (LPL100) LPL in the five dietary treatments. There were no significant differences in laying performance and egg quality among treatments. However, increasing LPL level showed linear effects on hen-day production (P = 0.009) and egg mass (P = 0.005). The hens fed LPL75 was greater hen-day production ($P < 0.05$), egg mass ($P < 0.01$), and yolk color score ($P < 0.05$) than those fed CON. Linear increase in dark yolk pigmentation was also observed for laying hens fed dietary LPL (P = 0.038). No significant differences were observed in the retention of dry matter, crude protein and ash. However, crude fat tended to improve with increasing levels of LPL (P = 0.051). Cholesterol fractions, vitamins A and E concentrations were unaffected by dietary treatments at 33-wk of age. However, linear effects were observed for triglyceride (P = 0.020) and vitamin A (P = 0.022) concentrations at 38 wks of age. The LPL100 lowered cholesterol in the blood compared to CON. Furthermore,

diets containing LPL100 had large percentage of C18:2n-6 and large ratio of polyunsaturated fatty acids to saturated fatty acids (SFA) than CON ($P < 0.05$). The supplementation of LPL also decreased the deposition percentage of SFA (linear effects; $P = 0.003$). Overall, the LPL can be used in laying hen diets to reduce cholesterol fractions and increase laying performance.

Experimental III. Effects of Metabolizable Energy Levels and Lysophospholipid Supplementation on Productive Performance, Egg Quality, Nutrient Retention, and Blood Metabolites of Laying Hens

This experiment was conducted to examine the effects of various metabolizable energy (ME) levels with or without lysophospholipid (LPL) supplementation on productive performance, egg quality, nutrient retention, and blood metabolites of laying hens. A total of 360 50-week-old Hy-Line W36 laying hens were subjected to 6 treatments in a 2 × 3 factorial arrangement with 2 levels of LPL (0 and 0.75 g/kg diet) and 3 levels of ME (2,670, 2,750, and 2,830 kcal/kg). Each treatment had 5 replications of 60 laying hens. No interactions were observed for all criteria of laying performance and egg quality. However, the main effect of the LPL significantly decreased egg weight and the cracked egg percentage ($P < 0.05$), as well as increased the yolk color score ($P < 0.0001$). The dietary ME levels did not improve nutrient retention of crude protein, but the LPL supplementation improved ether extract retention ($P < 0.05$) and a tendency for total ash ($P = 0.083$).

Serum concentrations of α-tocopherol, alanine aminotransferase, triglyceride, and high-density lipoprotein were not affected regardless of the interactions or the main factors during both periods. However, the interactions between ME levels and LPL supplementation significantly improved glucose concentration at 58 weeks of age, which reached the highest value in hens fed ME at a 2,750 kcal/kg combination with 0.75 g/kg of LPL supplementation ($P < 0.05$). Furthermore, the LPL addition showed an improvement in retinol ($P = 0.052$) and a reduction in cholesterol concentrations ($P = 0.075$). Overall, the supplementation of LPL alone can possibly be used in lower-ME diets to eliminate losses of laying performance and increase yolk pigmentation through the improvement in nutrient utilization.

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List of Abbreviations

AA	amino acids
ACAT	acyl-CoA cholesterol acyltransferase
ADG	average daily gain
ALT	alanine aminotransferase
AME_N	nitrogen-corrected apparent metabolizable energy
AOAC	Association of Official Analytical Chemists
ATTD	apparent total tract digestibility
BSA	bovine serum albumin
BW	body weight
BWG	body weight gain
CF	crude fiber
CMC	critical micellar concentration
CP	crude protein
CRD	completely randomized design
DHA	docosahexaenoic acids
EE	ether extract
ELIZA	enzyme linked immunosorbent assay
EPA	eicosapentaenoic acid

FA	fatty acids
FABP	fatty acid-binding protein
FAME	fatty acid methyl ester
FAO	Food and Agriculture Organization
FCR	feed conversion ratio
FFA	free fatty acids
FI	feed intake
FOS	fructooligosaccharides
GE	gross energy
GI	gastrointestinal tract
GlcNAc	*N*-acetylglucosamine
GLM	general linear model
GPR	glyceryl polyethylene glycol ricinoleate
HDL	high density lipoproteins
HLB	hydrophilic-lipophilic balance
IDL	intermediate density lipoproteins
IL-1	interleukin-1
IL-6	interleukin-6
LCFA	long chain fatty acids
LDL	low density lipoproteins
LPC	lysophosphatidylcholine

LPL	lysophospholipids
ME	metabolizable energy
MUFA	monounsaturated fatty acids
NC	negative control
ND	not detected
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PUFA	polyunsaturated fatty acids
RBL	rice bran lysolecithin
SCFA	short chain fatty acids
SEM	standard error of the mean
SFA	saturated fatty acids
TC	total cholesterol
TG	triglyceride
TNF-α	tumor necrosis factor alpha
VH:CD	villus height to crypt depth ratio
VLDL	very low density lipoproteins

Chapter I. General Introduction

Genetic improvements in bird performance and egg production have been gradually increased (Emmerson, 1997). Animal fats and vegetable oils are widely used in feed formulation to increase energy content so that growth performance and laying productivity can be improved and industry standard can also be achieved. Other advantages of fat supplementation include improved palatability and fat soluble vitamin absorption, decreased feed passage rate and controlled dustiness in feed mills (NRC, 1994). However, assimilation of dietary fats in young chicks is limited because they are insufficient to produce and secrete bile salts and pancreatic enzymes until the age of 10 to 14 days (Noy and Sklan, 1995). This limitation causes an inability to form mixed micelles in the intestinal lumen which further decreases the digestion and absorption of dietary fats (Leeson and Atteh, 1995). Furthermore, supplementation of fats as a high energy-yielding source has been an expensive component in poultry formula and will increase feed cost (USDA, 2015). The new strategy needs efficiently energy utilization in animal production.

Nutrients supply from crude protein and amino acids are important factors that affect growth and protein metabolism in growing broilers (Tesseraud et al., 1999; Hocquette et al., 2007). Becaused it is essential for supporting growth performance and poultry health (Scott, 2002; Sterling et al., 2005; Brickett et al., 2007). However, there has been recently considered that poultry producers should minimize nitrogenous nutrients in feed formulation in order to eliminate amount of excreted nitrogen release to the environment. It has been reported that the pollution with nitrogen originating from animal production has become a major problem in many countries (FAO, 2006). Most of animals, including poultry there are incompletely converting nitrogenous to tissue protein accumulation (Noy and Sklan, 1995). It is important that lower nitrogenous diets and improvement in the

efficiency of nitrogen deposition should be considered. Recently, researchers have paid attention on optimal growth rate and minimized nitrogen excretion by reducing crude protein content in feed formulation (Parr and Summers, 1991). However, this approach could not be achieved for high-yielding birds when nutrient is needed to support their growth (Holsheimer and Janssen, 1991; Moran et al., 1992).

The use of biosurfactants such as lecithin, lysolecithin (LC) and lysophospholipids (LPL) in poultry diet has shown to increase nutrient digestibility (Jansen et al., 2015). Previous studies showed beneficial effects of biosurfactants on improved energy utilization both in chickens and weaning pigs (Jones et al., 1992; Attia et al., 2009; Han et al., 2010; Jansen et al., 2015; Zhao et al., 2015). However, there are limited available data is known about the effectiveness of modified lecithin in reduced nutrient diets. The hypotheses for the present dissertation were that supplementation of LPL to broiler chickens and laying hens will improve their productivity and nutrient utilization, which subsequently enhanced intestinal integrity, anti-inflammatory and cholesterol-lowering effects. Consequently, a series of three experiments were conducted with the following objectives;

1) To investigate the effects of supplementation of lysophospholipids to low energy and nitrogenous diets on growth performance, blood metabolites, immunity, and gastrointestinal morphology in broiler chickens.

2) To investigate the optimum level of dietary lysophospholipids supplementation on laying performance, nutrient retention, blood related lipid metabolism, and yolk fatty acid deposition of brown egg-laying hens

3) To determine the effects of metabolizable energy level and lysophospholipids supplementation on productive performance, egg quality, and blood metabolites in laying hens.

Chapter II. Literature Review

1. Dietary Lipids in Poultry Diets

1.1 Definition and Classification of Lipids

Fat is referred to triglycerides or triacylglycerols of fatty acids which are poorly dissolved in water solution but highly dissolved in organic solvents (Gurr, 1997; Lehninger et al., 2008). It is higher gross energy approximately 2.25 times than carbohydrate (Leeson and Summers, 2001b). Pond et al. (2005) categorized dietary lipid into three groups, namely simple, compound and derived lipids.

1.1.1 Simple Lipids

Simple lipids refer to the esters of fatty acid along with various alcohols without any substances. Common simple lipid is triglycerides which contained three hydroxyl groups of glycerol.

1.1.2 Compound Lipids

Compound lipids refer to the ester of fatty acid containing alcohol, mineral, protein, and carbohydrate. Examples of compound lipids are phospholipids and lipoprotein cholesterols. The structure of phospholipids is mostly contained a phosphoric acid, nitrogen base and other substituents, whereas lipoproteins are contained both protein and lipid.

1.1.3 Derived Lipids

Derived lipids refer to derivative products of simple and compound lipids (fatty acids, glycerol, fat soluble vitamins, carotenoids, and steroids). They act as a component of cell membrane and use as a precursor of steroid hormone synthesis.

1.2 Functions of Lipid in Poultry Nutrition

Lipids play an important role in sustaining life for maintenance, growth, and production (Gropper et al., 2009). Excess amount of energy are then deposited in adipose tissues (Gurr, 1997). Published reports demonstrated that supplemental linoleic acid, originating from vegetable oils showed to increase egg weight (Scragg et al., 1987). An increased level of linoleic acid also increased egg mass (Pérez-Bonilla et al., 2011). The addition of lipids showed to improve fat soluble vitamin absorption was also reported by Han et al. (2010). Barbour et al. (2006) observed that laying hens fed crystalline carotene in a high fat diet modulated fat soluble vitamin absorption compared to those fed a normal fat diet. In broilers, dietary lipid had a great impact on feed intake it was observed when birds fed corn and soybean meal diets with no addition of lipid (Golian and Maurice, 1992). There was increased in feed intake of turkey poults during 3 to 11 days of age in group fed unsupplemented lipid compared to those fed lipids (Turner et al., 1999).

1.3 Lipid Digestion and Absorption in Poultry

1.3.1 Digestive Enzyme Activity

Endogenous digestive enzymes are involved in metabolic pathways of the digestion. The endogenous digestive enzymes can be categorized into two groups, pancreatic enzymes (α-amylase, protease, and lipase) and brush border enzymes (maltase, sucrase-isomaltase, and amino peptidase). In the intestinal lumen, α-amylase plays a vital role in hydrolyzed complex soluble polysaccharide to di- and monosaccharides, whereas protease plays a crucial role in metabolized polypeptides to single AA, di- and tri-peptides (Lanham-New et al., 2011). The disaccharides that penetrate into the microvilli are degraded by disaccharidase, yielding maltase, maltotriose and sucrase-isomaltase complex, whereas the di- and tri-peptides are

subsequently degraded by amino peptidase in the whole jejunal and ileal mucosa (Maroux et al., 1973; Galand, 1989). Uni et al. (1998a) demonstrated that the activity of disaccharidase is lowest in the duodenum, whereas the highest activity is found in the jejunal and ileal segments. In broilers, an improvement in sucrase and maltase activities were found in the upper half of villus, whereas inhibited activity is found in the lower half of villus (Uni et al., 1998b; Pinheiro et al., 2004). The change in intestinal disaccharidase might be inhibited brush-border enzyme secretions (Uni et al., 1998a; Chotinsky et al., 2001).

1.3.2 Nutrient Digestibility

The gastrointestinal tract is responsible for the digestion and absorption of nutrients, the net movement of nutrients is entered the gut to the blood circulation. Published research showed that a simultaneous movement of mucin, bile acids, bicarbonate, endogenous enzymes, electrolytes, sloughed cells and nitrogenous compounds from gut-associated tissues could also activate the net movement of N into the intestinal lumen (Fuller and Reeds, 1998). According to Clarke and Wiseman (2005), who demonstrated the activation of endogenous compounds in promising nutrient digestibility, representing energy cost due to the presence of trypsin inhibitor, and modulating endogenous secretions of amino acids (AA) and carbohydrates (Lien et al., 1997). This means that endogenous secretion and loss have a great impact on AA deposition (Piel et al., 2004). A number of poultry studies have explored the ways to improve nutrient digestibility. Recently, the use of biosurfactants in poultry study is being interested (Zhang et al., 2011; Mandalawi et al., 2015; Jansen et al., 2015). It showed to improve apparent total tract digestibility of crude fat (CF), fatty acids, crude protein (CP) and AA, reported in many studies (Attia et al., 2009; Han et al., 2010; Jansen et al., 2015).

1.3.3 Digestion and Absorption of Dietary Lipids

The digestion and absorption of fat are very unique because it requires chylomicrons to carry lipids (Krogdahl, 1985; Scott et al., 1982). The intact lipid is directly activated cholecystokinin, which consequently promotes lipase and bile salts secretions (Friedman and Nylund, 1980). Bile salts are well-established in aiding micelles formation because it increases surface area of lipid droplets for lipase and co-lipase to interact (Caspary, 1987). The major function of lipases is hydrolyzed triglycerides on *sn*-1 and *sn*-3 positions, yielding monoacylglycerol and free fatty acids (Friedman and Nylund, 1980). For cholesterol, cholesterol esterase is activated the hydrolysis of cholesterol-fatty acid and free fatty acids (Mu and Høy, 2004). The end products of fat digestion are short chain fatty acids (SCFA), long chain fatty acids (LCFA), monoglycerides, diglycerides, fat soluble vitamins, and cholesterol esters (Mu and Høy, 2004). The SCFA and monoglycerides are directly entered the intestinal lumen to mesentery blood vessels via intestinal cells. By contrast, some lipids are further hydrolyzed before entering intestinal cells (Davenport, 1980). These processes are needed bile salts for passing an unstirred water layer of the small intestine (Iqbal and Husain, 2009). Once monoglycerides and free fatty acids are entered intestinal lumen, re-esterified is occurred and required chylomicrons before releasing in the lymphatic vessels. However, the lymphatic vessel of poultry is not fully developed, therefore the chylomicrons are secreted to the portal circulation and protomicrons (Hermier, 1997). Protomicrons are transported mediator of chylomicrons in various tissues, especially in the liver (Scott et al., 1982). The absorption of fatty acids is required fatty acid-binding (FABP) to transport fatty acids in the cytosol (Ockner et a., 1972). Katongole and March (1979) noted that fat absorption is completed in the ileum due to the abundance of FABP. Published data also found that the FABP had greater affinity

to unsaturated than saturated fatty acids and lower affinity to medium and SCFA (Ockner and Manning, 1974). This implies that vegetable oils are more beneficial to increase fat absorption in poultry.

1.4 Partitioning of Dietary Energy in Poultry

Energy content in feed formulation is the first consideration because it presents as the largest portion. Partitioning of dietatry energy can be measured as calories (cal), kilocalories (kcal) or kilojoule (kJ) of gross energy (GE), digestible energy (DE), metabolizable energy (ME), and net energy (NE) (NRC, 1994).

The GE is defined as the heat release when a substrance is completely oxidized to carbon dioxide and water in a bomb calorimeter. The amount of GE in feedstuffs is varied depending on diet composition.

The DE represents differences between the GE of feed consumed and the GE of feces, urine, and gases. However, the GE of gaseous product is negligible in poultry. Also, the bird's excreta of feces and urine are difficult to separate. Therefore, the DE is only definded as the amount feed consumed minus fecal energy.

The ME is the deferrences between the GE of feed consumed and the GE of energy contained in the fecal and urinary products. The ME system is acceptable to identify energy requirement for poultry, and it is possibly applied to an apparent metabolizable energy (AME). The AME is the differences between the GE consumed and the GE contained in the excreta (feces, urine, and endogenous losses). The corrections for endogenous energy and N retained in animal body can be adapted to a true metabolizable energy (TME) and a nitrogen-corrected AME (AME_n), respectively. The equation for calculation of AME_n is; AME_n = AME - 34.39 x nitrogen retained in the body. However, the ME value is varied depending on dietary fat source of each ingredient (Table 1).

The NE can be categorized into two groups, the NE for maintenance

(NE_m) and production (NE_p). The NE_m is the energy required for basal metabolic processes, sustain animal life and body temperature. If the amount of NE is greater than the energy used for maintenance, it is required for NE_p. Therefore, the NE system is usually defined as the proportion of ME in feedstuffs minus energy lost as heat increment (HI). The HI is produced because of the lack of ability to complete digestion, fermentation and metabolic processes.

1.5 Metabolizable Energy and their Requirements in Broilers

Feed formulation for poultry is normally used the ME system rather than the NE system because it is simple and less expensive to determine AME (NRC, 1994). Wiseman and Salvador (1989) investigated the AME value of fats in broilers fed diets containing vegetable oil and beef tallow at 25, 50, 75, 100, and 125 g/kg at 2, 4, 6, and 8 weeks of age. Results found significant improvement in AME value of fats increased at the age of 2 to 4 weeks and remained unchanged onward. The improvement in AME of beef tallow was greater than vegetable oil, indicating highly attributed to fat emulsification for lipase activity.

Dozier et al. (2011) examined the AME_n of crude glycerine originating from different fat sources (soybean oil, tallow, and yellow grease) in male broilers Ross x Ross 708. Results showed that crude glycerine originating from soybean oil had similar percentage of AME_n (3,579 kcal/kg) of GE (98.44%) compared to those originating from tallow and yellow grease. However, it had a relative high amount of fatty acids which found to be poorly utilized by broilers.

Dozier and Gehring (2014) provided 6 levels of AME_n at 3,000, 3,030, 3,060, 3,090, 3,120, and 3,150 kcal/kg to diets for Hubbard x Cobb 500 and Ross x Ross 708 male broilers during 14 to 28 days of age. The results showed that feed conversion ratio (FCR) of Hubbard x Cobb 500 male broilers were responsed to the AME_n of 3,060 kcal/kg. Conversely, BW and FCR of Ross x Ross 708 broilers did

not response to the change of 3,000 kcal of AME_n/kg to 3,150 kcal of AME_n/kg. There are several factors that influence energy partitioning such as age, gender, strain, level of intake, physiological status and feed form. Published research has shown that newly hatched chicks are unable to complete digestion and absorption of dietary fat due to the immature of the gastrointestinal tract and lower secretion of pancreatic lipase (Krogdahl, 1985). Noy and Sklan (1998) reported that secretions of lipase, trypsin, and amylase in the duodenum increase about 20 to 100 times between days 4 and 21 post hatch. However, the production of pancreatic lipase was found to be slower than other digestive enzymes. In addition, the synthesis of fatty acid binding protein (FABP) has been reported to be insufficient in young birds, but gradually increased after 4 week of age. Recently, Tancharoenrat et al. (2013) investigated the influence of age on AME in broiler chickens during weeks 1, 2, 3 and 5 posthatch. Results showed that the AME was lower during 1 week posthatch, but increased during 2 week posthatch.

1.6 Metabolizable Energy and their Requirements in Laying Hens

Laying hens normally store energy after their maintenance and production are met. The energy is initially needed for maintenance, followed by lean protein accretion and fat accretion, respectively. If the energy needed for maintenance is not satisfied, the birds will over consume nutrients or catabolize storage energy to replace those in the diet (Sakomura, 2004). Valkonen et al. (2008) observed the effects of various ME levels on laying performance of two caging systems (8-hen furnished cages vs. 3-hen conventional cages). A total of 1,088 Lohmann Selected Leghorn were fed the diets during three feeding programs of 16, 18, and 20 weeks of age. The experimental diets were low ME (2,342 to 2,414 kcal/kg) and high ME (2,581 to 2,629 kcal/kg). Results showed that feed intake of hen fed low energy diet was significantly increased about 8 to 9 g/hen/d more than those fed the

high energy diet ($P < 0.01$). However, hens in group fed low energy diet produced less egg production than those consumed high energey diet ($P < 0.05$). Hussein et al. (1996) determined the effects of a high energy (3.09 Mcal AME_n/kg diet) and a low energy (2.78 Mcal AME_n/kg diet) on pullet development of Single Comb White Leghorn from weeks 15 to 18. Results showed there was a significant increase in egg weight in the high energy and a reduction in feed consumption. However, mortality percentage, 50% of egg production, egg production, and egg weight were not influenced by dietary treatments. In Pearl Gray guinea fowl pullets from 0 to 8 week of age, feeding the energy level at 3,000 and 3,100 kcal of ME/kg had positively increase BW gain compared to group fed 2,900 kcal of ME/kg. In addition, the improvements in egg production, egg mass, egg quality were greater in pullets fed 3,000 and 3,100 kcal of ME/kg diets than those fed 2,900 kcal of ME/kg at 0 to 8 week of age (Nahashon et al., 2007). Feed conversion ratio in birds consumed 3,000 and 3,100 kcal of ME was decreased at 1 to 5 and 6 to 8 week of age. However, feed intake of birds fed a low energy diet at 2,900 kcal of ME was significantly higher than other treatments (Nahashon et al., 2006).

D'Alfonso et al. (1996) observed that different levels of ME at 2,580, 2,814 and 3,009 kcal/kg in De-Kalb XL laying hens did not affect egg production, egg mass, and body weight change, but a linear decrease was detected with the increased ME level. However, the results of lower ME diet on hen's performance are still controversial. For instance, feeding various ME levels, originating from corn oils at different levels (2,783, 2,891, 2,996 and 3,089 kcal/kg) or poultry fat (2,881, 2,975 and 3,059 kcal/kg) for a 12-week feeding period. There were unaffected on average daily feed intake of 560 Hy-Line W36 laying hens when reduced ME (2,996 to 2,783 kcal/kg) was fed (Bohnsack et al., 2002). This study was consistent with Jalal et al. (2006), who observed that Hy-Line W36 laying hens did not show negatively responses to the low ME diets (2,800, 2,850 and 2,900

kcal/kg). Furthermore, the ME was unaffected on feed intake, egg production, egg mass and egg weight among treatments. Harms et al. (2000) observed the effects of energy level on egg production using four laying hen breeds (Hy-Line Brown, Hy-Line W98, Hy-Line W36, and DeKalb White) when conducted the experiment from 36 to 44 weeks of age. Treatments were low ME (2,519 kcal/kg), moderate ME (2,798 kcal/kg), and high ME (3,078 kcal/kg). Results showed that laying hens fed the low energy diet consumed 8.5% more feed than those fed the moderate energy diet. Conversely, the high energy hens consumed 1.5% less feed than hens fed the moderate energy. Hens from four commercial strains are more sensitive to the changes in energy. The authors explained that the Hy-Line Brown and Hy-Line W98 hens were susceptible to the changes of energy level than the Hy-Line W36 and DeKalb White hens due to their genetic improvement. However, hen-day egg production was not affected regardless of ME supplementation but egg weight slightly increased by 2% in the high ME diet. At 80 week of age, the Hy-Line W36 hens was not responsed to energy level (2,519 to 2,798 kcal/kg) because feed intake and hen-day egg production remained unaltered over an 8-week feeding (Harms and Russell, 2004).

It seems that egg production is unaffected during the short experimental periods. According to Murugesan and Persia (2013), who conducted the experiment using 60 Hy-Line W36 laying hens for a 12-week feeding to examine the effect of ME levels (2,790 and 2,880 kcal/kg) on laying hens' performance. Results showed a significant effect on feeding program in which feed consumption in the *ad libitum* consumed 10 g/hen/d more than restricted group ($P < 0.01$). Percentage of hen-day egg production in the *ad libitum* was 3.5% higher than the restricted hens ($P < 0.01$). The positive effect of the high energy diet in the *ad libitum* also improved egg mass, increased abdominal fat pad and body weight (BW), increasing by 3.5% g/hen/day for egg mass, 80 g heavier in BW, and 23.8 g/hen in relative abdominal fat weights.

However, the differences of energy intake between two experimental diets did not influence egg production, but decreasing ME level showed lowered accumulation of the abdominal fat pad.

Table 1. Apparent metabolizable energy in various fat sources of young and adult birds

Fat source	Mcal/kg	Fat level (g/kg)[1]	Age (day)	Reference
Starter to grower chickens				
Poultry fat	38.02	70	13-17	Lessire et al. (1982)
Beef tallow	29.35	70	13-17	Lessire et al. (1982)
Beef tallow	27.35	20	16-19	Wiseman et al. (1986)
Beef tallow	26.02	90	13-24	Huyghebaert et al. (1988)
Beef tallow	31.31	40	14-18	Blanch et al. (1996)
Palm oil	24.56	90	13.24	Huyghebaert et al. (1988)
Palm oil	27.07	60	9-10	Pesti et al. (2002)
Soybean oil	40.53	20	16-19	Wiseman et al. (1986)
Soybean oil	35.69	90	13-24	Huyghebaert et al. (1988)
Soybean oil	46.48	60	9-10	Pesti et al. (2002)
Adult chickens				
Poultry fat	37.35	70	40-44	Lessire et al. (1982)
Beef tallow	30.60	70	40-44	Lessire et al. (1982)
Palm oil	21.88	60	39-40	Pesti et al. (2002)
Soybean oil	40.00	60	39-40	Pesti et al. (2002)

[1]Level of fats used in the experimental diets

2. Gastrointestinal Tract of Chicken Post-hatch

2.1 Development of the Gastrointestinal Tract (GI)

Egg yolk is important nutrient for new-hatching chicks before giving exogenous feed such as carbohydrate and proteins (Uni et al., 1999; Sklan, 2001). Many available reports have shown that relative weights and lengths of the intestinal segments of broilers are rapidly changed in a starter period from days 6 to 10 (Sklan, 2001; Iji et al., 2001a; Noy et al., 2001; Uni et al., 2003). Previous research demonstrated that the development of intestinal tract in new-hatch chicks reached a peak at 14 d of age and decreased substantially thereafter (Uni and Ferket, 2004). However, the result was still controversial, for example Iji et al. (2001a) observed that age of chick influenced the decreases in body weight, gizzard and yolk residual.

2.2 Factors Regulating Morphology Development in GI Tract

2.2.1 Stressors

External stressors such as temperature (heat and cold stress), infectious disease, and high stoking density cause detrimental impacts on morphological development of the small intestine, especially changes in villus height and crypt depth. Heat stress is one of important critic issues in poultry production, particularly in tropical countries. It can be defined when the high ambient temperature is increased than the thermo neutral zone which resulted in increased intestinal colonization and fecal shading of infectious pathogens (Bailey, 1988). The heat stress also affects microbial populations and intestinal mucosa of intestinal segments in broilers (Burkholder et al., 2008). Results observed that birds exposed to high temperature above 30 ^{o}C for 24 h, significantly increased ileal crypt depth in comparison to those raised in a neutral temperature (23 ^{o}C). However, no differences were detected for villus height and ratio of villus height to crypt depth

(VH:CD) were observed when the temperature increased. One of the examples is high stocking density. Puron et al. (1995) showed that small space allowance contributed to longer crypt depth and shorter villus height. It is possible that under the high stocking density, it reduced airflow and increased ammonia leading to reduced feed intake (Burkholder et al., 2008).

2.2.2 Nutritional Aspects

There are many available publications of diet manipulation in inhibiting intestinal morphology such as feeding diets containing anti-nutritional factors and mycotoxin contaminations as well as protein level. According to Swatson et al. (2002), who demonstrated that increased utilization of protein influenced intestinal mucosa of crypt and development of villus height. For instance, increased crypt depth can be assessed as an incidence of pathogenic invasion in which new intestinal cells are formed, whereas increased villi height can be determined the increased absorptive area for better nutrient absorption. According to Yamauchi (2002), the intestinal segments of broilers can be modified depending on the present of digested nutrients in the small intestinal lumen. Maneewan and Yamauchi (2003) observed that semipurified protein-free diets had the slowest in promoting histological recovery after feed withdrawal, suggesting that protein level plays a role in recovery of the gut morphology. Furthermore, Laudadio et al. (2012) found that decreased dietary protein level less than 18.5% in isoenergetic diets could suppress growth performance of broilers compared to those fed high-22.5% and medium-CP (20.5%) diets. Khonyoung et al. (2015) pointed out that decreased villus height and VH:CD were associated with lowered nutrient absorption. Shorter villus is a marker to identify the suppression of luminal villus absorptive area and subsequently inhibit enzyme function and nutrient transport. Furthermore, the lower VH:CD in broiler's fed the medium-protein level leading to increase

turnover of the intestinal mucosa. A higher turnover rate is considerable to increase nutrient requirements for maintenance, which finally leads to growth retardation (Burkholder et al., 2008).

2.3 Factors Affecting Intestinal Morphology in Poultry

2.3.1 Feed Additives

Intestinal development after hatch is rapidly changed with respect to enzymatic and absorptive activities (Yegani and Korver, 2008). The small intestine of young chicks is not fully mature and undergoes morphology, biochemical, and molecular changes during the first 2 wk posthatch. There are many factors that affect gut health such as prebiotics, probiotics and exogenous enzymes (Tsirtsikos et al., 2012). Wang et al. (2005) observed the effect of enzyme supplementation primary xylanase and β-glucanase with 0, 200, 400, 600, 800, and 1,000 mg/kg in a wheat-based diet during 7 to 42 d of age. Results observed the optimum enzyme level was 200 mg/kg diet. However, increasing enzyme level showed to decrease relative weight and length of ileal due to increased the viscosity of the digesta and inhibited the interaction between the digestive enzymes and their corresponding substrates. It resulted in modification of intestinal structure and function.

For prebiotic trials, feeding fructooligosaccharides (FOS) at 0, 2, 4, and 8 g/kg for male broiler was reported by Xu et al. (2003). The result found that inclusion of 4 g/kg FOS enhanced intestinal microbial flora. The ileal microvillus height, villus-height-to-crypt-depth and digestive enzyme production also increased. Furthermore, at the inclusion of 0.5% FOS had promising impacts in modulating intestinal mucosa and ant-inflammatory action in comparison to those fed diets with no supplemented treatment (Shang et al., 2015). Alternative strategy of feed alternatives using natural surfactant has recently reported by Khonyoung et al. (2015). The author found that supplemental lysolecithins with different fat sources

significantly increased duodenal cell mitosis mitosis with 0.23 cell/μm^2 compared to control birds with 0.019 cell/ μm^2 of protuberated cells

2.3.2 Optimum Nutrient Requirement

A number of studies have invested the effect of optimum nutrient requirements on broilers performance and morphological structure. For instance, Laudadio et al. (2012) observed that the birds fed diet containing 20.5% CP could maximize growth rate as similar to those fed 22.5% CP-supplemented group, in accordance to improved intestinal health with no detrimental effect on meat quality. Buwjoom et al. (2010) assessed that long-period feeding of low-CP diet at 10%, 16%, and 22% CP did not show significant effects on villus height and villus area of all intestinal segments.

Wang et al. (2015) showed that broilers fed different protein sources and nutrient density showed morphology impacts. For example, the birds fed high protein diets had shorter small intestine, whereas higher nutrient density was longer jejunal villus contributing to improve nutrient absorption. Moderate amounts of fiber also had the impacts on digestive organ development, enzyme secretion, and nutrient digestion in birds (González-Alvarado et al., 2007). These improvements are mostly contributed to the increasing gastroduodenal refluxes for activating the incorporation between digestive enzymes and nutrients (Duke, 1992).

2.4 Morphological Development of the GI tract

The GI tract consists of several anatomically and functionally distinct segments such as duodenum, jejunum, ileum, ceca and colon. A well-functioning GI tract has to transport nutrients from the intestinal lumen into the blood circulation, while increasing the resistance to the bacterial invasion. In addition, the GI tract plays a significant role in saving host energy, protecting barrier

shedding the body from foreign substances (Duke, 1992).

2.4.1 Villus Height, Crypt Depth, and Rate of Epithelial Turnover

The GI tract of chickens is immediately changed after hatching. Increasing the surface area for digestion and absorption is an important step for the chicks to express their performance (Nitsan, 1995). Intestinal development is important to determine nutrient absorption through promoting intestinal diameter and consequently affect relative weight of intestinal segment (Gomide et al., 2004). The high integrity of the intestinal mucosa is unique feature that allows for the response to foreign agents such as presence or absence of feed and bacterial invasion. Examples response consists of changes in villus height, crypt depth, epithelial turnover (Gomide et al., 2004).

Intestinal development of morphological structure such as villus height, crypt depth and ratios of crypt to villus height is an indicative measurement of gut status. The improvement in villus height and villus height to crypt depth ratio for various gut segments indicate an increase absorptive area and subsequently result in promoted digestive enzyme function (Coates et al., 1955; Tufarelli et al., 2010). These morphological developments can promote growth performance due to better nutrient digestion and absorption. Bartell and Batal (2007) indicated that increased surface is possibly associated with heavier intestinal segments of the small intestine. For example, higher ratios of villus height to crypt, indicating a decrease turnover rate of intestinal mucosa caused by pathogen infections. Van Nevel et al. (2005) pointed out that a slower turnover rate of the intestinal epithelium caused subsequently effects on improved growth rate and feed efficiency. By contrast, a longer crypt depth indicates bacterial or toxin infections by increased renewal of the villus, that animals are attempted to compensate for normal sloughing or atrophy of villi (Yason et al., 1987; Xu et al., 2003; Gao et al.,

2008). Impaired intestinal morphology may influence nutrient utilization, growth performance, and animal health status. Many researchers have shown that dietary manipulation has a great impact on intestinal development of the epithelial cells during bacterial infection (Petronini et al., 1992; Persia et al., 2006).

The crypt of the villus contain several specialized cells such as absorptive cells, goblet cells, and regenerative cells that play a crucial role in the production of mucus and the replacement of old intestinal cells (Solis et al., 2005). It is well-establish that Lieberkuhn plays an essential role in intestinal epithelium, mostly migrate up the sides of the villus (Imondi and Bird, 1966). In young birds, it is occurred every 90 to 96 h (Solis et al., 2005). Previous research has showed that the development of villus and crypt undergoes rapidly change during the first few days after hatching (Marcari, 1998). The villus height in the small intestine increases with age of bird (Uni et al., 1999). The absorptive area is dramatically changed until 21 days, which is larger in the duodenum than the jejunum or ileum (Iji et al., 2001a). According to Geyra et al. (2001), the development of the intestinal mucosa and enterocyte activity changed until 12 d of age in newly hatching chicks. At hatch, intestinal epithelial exhibited round or unshaped polar with no difference in villus formation. During 24 h post-hatch, villi were extended which comparable to mature intestinal morphology in all intestinal compartments. The villus length was then gradually increased, which reached a plateau on 9 d post-hatch in the duodenum, 6 d post-hatch in the jejunum, and 1 d post-hatch in the ileum. The absorptive areas in the jejunum and ileum segments are developed slightly in comparison to those in duodenum at 4 d post-hatch upward, whereas the total segment of villus absorptive area developed similarly in all segments by 3 d post-hatch (Geyra et al., 2001). Uni et al. (1999) observed that enterocyte size increased slightly in the initial phase of post-hatch, and reached a peak almost d 6, especially in the duodenum. The authors found the peak of villus height and crypt

depth in the jejunum and ileum was on d 10 after hatch. It is indicates that the maturation of intestinal morphology with specific ontogenetic development is varied depending on various compartments of small intestine. The longer villus height or shorter crypt depth is associated to promote absorptive capacity or susceptible inflammatory response in the small intestine (Uni et al., 1999). Consequently, enhanced development of intestinal mucosa may have a primary modulation in the mechanisms of nutrient utilization and subsequently effect on broiler performance.

3. Emulsifier

In feed manufacturing, emulsifiers can be categorized into two groups, namely natural emulsifiers and synthetic emulsifiers. Details are given with the following orders;

3.1 Natural Emulsifiers

Natural emulsifiers can be produced in animal body (phospholipids and bile acid) or provided from plant origins. Vegetable lecithins, containing primarily phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositiol are derived commercially from oil seeds such as soybeans (Soares and Lopez-Bote, 2002) and rapeseed (Raju et al., 2011). The lecithins are widely used as effective emulsifiers in various foods, pharmaceutical and technical applications (Van Nieuwenhuyzen and Tomás, 2008). Details of each emulsifier are provided as the following points;

3.1.1 Bile Acids and Bile Salts

Bile is excreted fluid of the liver which contained bile pigments (biliverdin), bile salts, phospholipid, cholesterol, electrolytes, and several proteins (Krogdahl, 1985; Hofmann, 1994). Bile is well-established as an effective natural

emulsifier because the structure of the bile is very unique. It is composed of two components, a hydrophobic tail for lipid interaction and a hydrophilic tail for water interaction (Chen et al., 1975). These characteristics are powerful in catabolic metabolites of bilirubin, emulsified fat-soluble vitamins to enable their absorption, and reduce bacterial population in the small intestine as well as in the biliary tract.

Secretion of bile is considerable to be insufficient in newly hatch poultry and during early development stages, resulting in reduced fat digestion and absorption (Krogdahl, 1985). Additionally, the replenishment of bile salts in young birds is lower than old birds (see Table 2). Decrease in pool size of bile salts may also contribute to poor digestion and absorption during the first few weeks of bird's life (Serafin and Nesheim, 1970). For these reasons, the addition of bile salts such as bile acid derivatives (bile salt and cholic acid) to poultry diets has been investigated.

Table 2. Concentrations of bile acids in young chicken and duck (mg/g)

Bile acid	Chicken	Duck
Cholic 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)

Gomez and Polin (1976) studied the absorption of saturated fat (tallow) in chicks from 4 to 7 and from 14 to 19 days of age by providing bile acids (cholic acid and chenodesoxycholic acid) and bile salts (taurocholate) at 0, 0.25 and 0.50 g/kg to a corn-soy diet with 82 g/kg tallow. Results showed birds fed bile salts and bile acids increased fat absorption in both periods in comparison to unsupplemented treatments. Furthermore, the addition of cholic acid showed a better improvement in tallow absorption than chenodeoxycholic acid and taurocholate. However, supplementation of bile was less effective in fat absorption for aged laying hens. Polin et al. (1980) also found the positive improvement of bile acids on lipid absorption of tallow when added at 0.4 g/kg cholic acid in chicks during 0 to 7 days of age compared to unsupplemented group. At 21 day of age, the birds in the cholic acid group showed an enhancement in fat absorption compared to those fed unsupplemented group (87.1 versus. 84.8%) and chenodeoxycholic acid group (87.1 versus. 81.0%). In addition, Atteh and Lesson (1985) observed that the supplementary cholic acid in diet containing mixture of palmitic acid and oleic acids resulted in better performance of broiler chickens through the improvement of ME.

Different bile sources have been intensively studies to improve animal performance. For examples, Orban and Harmon (2000) found the improved fat digestibility in weaning pigs fed desiccated pig powder. Alzawqari et al. (2011) also showed the effects of inclusion level of desiccated ox bile at 5 g/kg had greater weight gain and lower feed conversion ratio than 2.5 and 0 g/kg ($P < 0.05$) in broilers fed tallow diet. Fat digestibility in birds fed 5 g/kg bile treatment (78.9%) was significantly higher than those fed diets containing 0 and 2.5 g/kg dried ox bile (52 and 69%, respectively) in a starter period by activation of morphological maturation of the gastrointestinal tract.

3.1.2 Lecithin

Lecithin is a mixture of natural phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), which are purified extracted during the processing of soybean oil through the separation from the oil by addition of water and centrifugation or steam precipitation (Wendel, 2000; Van Nieuwenhuyzen and Tomás, 2008). Soybean lecithin is widely used as a natural stabilizer in various food applications. Because it has a hydrophilic portion with affinity for fats, and a hydrophobic portion with affinity to water (Gu and Li, 2003). According to Van Nieuwenhuyzen and Tomás (2008), vegetable lecithin has an excellent source of essential fatty acids (Table 3) and has a high metabolic energy content of 8 kcal equivalent to 34 KJ/g. Additionally, it contains high amount of the organic bound choline and phospholipids which could be used for optimizing feed quality and improving antioxidant properties.

Numerous studies have been studied the effect of lecithin inclusion on fat digestibility in young animals (Polin et al., 1980; Overland et al., 1993a; Soares and Lopez-Botes, 2002). According to Polin (1980), the supplementary lecithin at 0.2, 2, and 20 g/kg in broiler's diet containing 40 g/kg tallow. Results demonstrated an increased nutrient digestibility in broilers fed 20 g/kg lecithin in comparison to those fed 0.2 and 2 g/kg lecithin. Azman and Ciftci (2004) substituted dietary fat sources (soybean oil and beef tallow) at different ratios with lecithin on broiler performance. From 5 to 21 days of age, broilers were consumed diets containing 40 g/kg soybean oil (control), and 40 g/kg soybean oil and soy-lecithin mixtures (in 75:25 and 50:50 proportions) or 40 g/kg beef tallow and soy-lecithin mixtures (in 50:50 proportions). Results showed the birds fed tallow-lecithin combination diets significantly lower feed conversion ratio (FCR) than those fed soybean oil and soybean oil-lecithin mixture. However, there was not observed difference in FCR of birds fed either soybean oil or soybean oil in combinations with soy-lecithin.

Table 3. Fatty acid and phospholipid compositions from plant origins

Amount (%)	Soybean	Sunflower	Rapeseed
Fatty acids			
C16:0	16	11	7
C18:0	4	4	1
C18:1	17	18	56
C18:2	55	63	25
C18:3	7	0	6
Others	1	4	5
Phospholipids			
Phosphatidylcholine	15	16	17
Phosphatidylethanolamine	11	8	9
Phosphatidylinositol	10	14	10
Phosphatidic acid	4	3	4
Other phospholipids	7	6	6
All phospholipids	47	47	46

Source: Van Nieuwenhuyzen and Tomás (2008)

3.1.3 Lysolecithins

Lysolecithins (LC) are also known as lysophospholipids (LPL) which purified from soy-lecithin by an enzymatic conversion of phospholipase A_2. During the production of LC, a phospholipase plays a role to remove one of fatty acids from the phospholipids at *sn*-1 and *sn*-2 positions (Joshi et al., 2006). During this process, the phospholipids are then converted into LPL. The LC is therefore a mixture of phospho- and LPL which differ in phosphatidyl substituent and fatty acid pattern (Wendel, 2000; Joshi et al., 2006; Van Nieuwenhuyzen and Tomás, 2008). The structure of LPL has a polar head (phosphatidyl substituent)

and a single fatty acid chain which is different from common phospholipids. The polar head can be a choline, an ethanolamine, a serine, or an inositol. Due to the removal of one fatty acid, LPL are more hydrophilic and therefore have better oil-in-water emulsifying property than regular phospholipids (Joshi et al., 2006; Liu and Ma, 2011). Based on this characteristic, the LPL is able to possess the permeability of cell membrane and work together with bile salts during the first stages of fat digestion.

3.1.4 Lysophosphatidylcholine

Lysophosphatidylcholine (LPC) is a monoacyl derivative of phosphatidylcholine, which produced by the reaction of phospholipase A_2 activity. It is known to be an effective natural emulsifier because it has a critical micelle concentration (CMC) of 0.02 to 0.2 mM/L, which is about 20 to 200 times smaller than bile (CMC = 4 mM/L) and lecithin (CMC = 0.3 to 2 mM/L) (Langmuir, 2002). It has a major impact in forming spherical micelles in aqueous solution of the GI tract (Vasanthakumari et al., 2011). An observation of Zhang et al. (2011) demonstrated that supplementation LPC at 0.5 g/kg diets with three fat sources (soybean oil, tallow, and poultry fat) at 30 g/kg in starter diets and at 40 g/kg in grower diets of broiler chickens. There was increased bird BW gain in the starter, increased apparent metabolizable energy (AME_N) in the grower, and tended to reduce FCR in the starter periods. The highest value of AME was determined for birds fed emulsified diets with poultry fat. The improvements in broiler BWG may associate with greater apparent total tract digestibility (ATTD) of C18:2 and C18:3-*n*3 owing to LPC supplementation.

3.2 Synthetic Emulsifiers

Recently, a number of synthetic emulsifiers are available due to cheaper

price than those from biosurfactant. Examples are lysophosphatidylcholine (Lysoforte^{TM}, Kemin Industries, Sigapore), lysolecithin (AD. Emulsifier, Ad. Biotech, New York, USA) and glycerol polyethylene glycol ricinolate (Volamel Extra, Nukamel Inc., Hoogbuul, Olen, Belgium).

Glyceryl polyethylene glycol ricinoleate (GPR) or polyethylene glycol castor oil is mostly produced from ethylene oxide and castor oil. Castor oils itself is a triglyceride extracted from the seeds of the plant *Ricinus communis* and comprising mainly ricinoleic acid more than 85% with minor amounts of palmitic, oleic, linoleic, dihydroxystearic and arachidonic acids. The structure of GPR contains complex mixtures of various hydrophobic and hydrophilic components. The hydrophobic constituents include fatty acid esters of polyethylene glycol, whereas hydrophilic part consists of polyethylene ricinoleates, ethoxylated glycerol and polyethylene glycols (EURL, 2013). Roy et al. (2010) demonstrated that supplemented GPR at 10 g/kg of saturated palm oil improved broilers' live weight by up to 5% and, in this approach decreased FCR. It also found to increase fat and nitrogen utilization. According to Kaczmarek et al. (2015), the influence of GPR (without addition or added at 4 g/kg diet) in two levels of nitrogen-corrected apparent metabolizable energy (AME_N) at standard energy requirement or energy reduction by 0.4 MJ/kg diet on broilers' performance and ATTD. Results showed birds fed diets supplemented with GPR had higher BWG and lower FCR than birds consumed diets without GPR. Furthermore, the GPR inclusion to low-AME_N diet could improve ATTD of neutral detergent fiber of wheat-maize-soybean meal at 35 day of age.

3.3 Biosurfactants Action for Integral Membrane Protein

The structure of cell membranes is known as a lipid bilayer because it consists of lipophilic and non-lipophilic components (Spector and Yorek, 1985). It

is important for regulating nutrient movements (Myher et al., 1989). Lundæk and Anderseen (1994) showed the effects of LPL in modifying membranous proteins including ion channels. Result observed that various LPL supplementations (lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylserine and lysophosphatidylinositol) could alter the gramicidin channel. The changes in deformation energy of cell membrane by opening gramicidin channel, resulted in grater influx of macromolecules across the cell membrane. The alterations in energy change may constitute a general mechanism whereby the host bilayer can affect protein structure that will alter the hydrophobic interface between the protein and the surrounding bilayer. Such changes have been described for gap junction channels in order to open or close the channel for facilitate both lipophilic and non-lipophilic substances. The authors pointed out that the coupling between integral membrane proteins and their surrounding lipid bilayers can give rise to substantial interaction energies, which will contribute to the overall energy changes between different states of the protein. Recently, Lundaek et al. (2010) observed that changes in lipid compositions such as cholesterol, polyunsaturated fatty acids, other lipid metabolites and amphiphiles also altered bilayer physical properties.

3.4 Previous Studies of Biosurfactant Emulsifiers

Use of biosurfactants such as phospholipids, lecithins, and lysolecithin in feed formulation has been reported in many studies (Poilin et al., 1980; Al-Marzooqi and Leeson, 1999; Soares and Lopez-Bote, 2002). Available data are listed in the following aspects;

3.4.1 Laboratory Animal Studies

Koo and Noh (2001) showed that infused phosphatidylcholine (PC) to male rats at 3.0 mL/h for 8 h significantly increased α-tocopherol absorption

compared with those in control. There was increased by 42 to 43% in male rat infused with PC compared with that in unsupplemented-PC rats. Additionally, Sugawara et al. (2001), who used differentiated Caco-2 human intestinal cells to investigate carotenoids absorption in mixed micelles. Results showed that the uptake of micellar β-carotene and lutein was suppressed by PC supplementation in a dose response manner, whereas lysophosphatidylcholine (LPC), the lipolysis product of enzymatic hydrolysis (phospholipase A_2), significantly promoted the vitamin uptake of β-carotene and lutein, as well as epoxy carotenoids such as violaxanthin, neoxanthin, and fucoxanthin from micellar carotenoids. Baskaran (2003) also shown the combinations of PC and LPC on improved β-carotene and lutein accumulations, observed in plasma and liver. These positively effects are essential in increased nutrient uptake of carotenoids to form micelles.

Tagesson et al. (1985) showed that LPC increased ileal permeability to intestinal absorption of macromolecules when rats subjected to pathogenesis of many diseases, especially when involving allergic reactions to engulf foreign materials. The authors determined permeability changes of the intestine in rat using larger dextrans (3000 to 70 000 daltons) and bovine serum albumin (BSA) with or without LPC. Results showed that LPC at 20 mM damaged the morphological of the ileal mucosa, and 0.01 to 1 mM LPC induced *N*-acetyl-β-glucosaminidase activity. Suggesting that a natural surfactant of LPC might damage mucosal cells and release lysosomal enzyme activity, and that higher LPL concentration increase the shedding of enterocytes at the villus tips and augment the mucosal permeability to dextran 70 000 and BSA.

Several reports have showed that dietary phospholipids are beneficial in cholesterol-lowering effect when fed in hypercholesterolmic diets to rat, guinea pigs and monkeys. According to a result of Murata et al. (1983), who demonstrated the hypocholesterolemic effect by feeding phospholipid contributed to decrease

cholesterol secretion and promote high density lipoproteins (HDL) uptake into the liver. In rats, supplementation lecithin significantly reduced the secretion of lipoprotein A-1 and cholesterol from the liver. Kabir and Ide (1995) also showed that dietary soybean lysophospholipids reduced the activities of regulating enzymes involving in fatty acid synthesis. Ide et al. (1992) noted that rats fed phospholipid-supplemented diet decreased liver diacylglycerol.

Jimenez et al. (1990) found that adult rats fed a hypercholesterolemic diet (25% saturated fat, 1% cholesterol, and 0.5% cholic acid) containing various levels of purified polyunsaturated lecithin (2.5 or 0.7%) could reduce very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) cholesterols, and increased HDL. The reduction of unesterified cholesterol fractions to the liver may be modulated and directed towards to the bile for further excretion. According to LeBlanc et al. (2003), who investigated the mechanism of lecithin-induced for decreasing VLDL concentration via the inhibition of acyl-CoA cholesterol acyltransferase (ACAT). The result showed a dramatic decrease of 75% microsomal ACAT activity. This indicates that hepatic bile formation and secretion are active in regulating cholesterol homeostasis because ACAT plays an important role in inhibiting VLDL secretion (Suckling and Stange, 1985; Burnett et al., 1999). It functions in stimulating bile formation and secretions of biliary bile acid, phospholipid and cholesterol (Polichetti et al., 1996; Rioux et al., 1997). In hypercholesterolemic rabbit, lecithin-rich diet also induces a decrease of beta-VLDL cholesterol and of beta-VLDL-triacylglycerol while enhanced biliary lipid output (Polichetti et al., 2000).

3.4.2 Human Studies

Soybean lecithins are beneficial for patients in terms of lipid-lowering cholesterol and apoptosis. For example, oral administration of lecithin granulate at

1 teaspoon (73% phospholipids and approximately 23% of other important active ingredients) three times daily before meals for 30 days to patients with primary hyperlipidemia. Results showed lecithin was an effective material in lowered cholesterol and triglyceride concentrations. The reduction of total cholesterol diminished by 33% and LDL concentrations diminished by 38%, whereas the concentration in HDL-cholesterol and HDL-phospholipid increased markedly about 46% (Wójcicki et al. 1995). This may be explained by increasing HDL fractions from tissues including smooth muscle cell in the aorta wall and facilitating cholesterol transport to the liver, therefore preventing the deposition and the formation of atheromatous plaques.

3.4.3 Swine Studies

Cera et al. (1990) noted that digestibility of fat in pigs gradually increased after postweaning. The effects of lecithin supplementation on growth performance and digestibility of young pigs are controversial and limited (Jones et al., 1992; Overland et al., 1993ab). For example, Jin et al. (1998) found that addition of lecithin at 100 g/kg diet in tallow diets for weaned pigs increased average daily gain by 7.2% compared to unsupplemented lecithin group. The addition of lecithin also increased digestibility of gross energy, dry matter, ether extract, and crude protein, which was similar to Polin (1980) and Jones et al. (1992) in which the inclusion of lecithin increased digestibility of fat sources with long chain saturated fatty acids. The remarkable improvement in tallow digestibility by lecithin supplementation may increase the size of bile salt micelles, therefore increasing the interior capacity to incorporate more fatty acids. However, Jones et al. (1992) examined the supplementations of lecithin and lysolecithin for weanling pigs with different fat sources (soybean oil, coconut oil, tallow, and grease). Results observed that digestibility of tallow and lecithin blends was comparable to that of soybean oil and

tallow without lecithin. This was similar to the observation of Soares and Lopez-Bete (2002), who did not observe the effect of lecithin in weaning pig diets containing lard on digestibility of dry matter (DM), CP, and CF. The discrepancy is likely attributed to differences in diet compositions including variations in lecithin content.

Encouraging research on biosurfactants in livestock animals has been increased. For instance, the effect of soy-derived lecithin at 10 g/kg diet has positive effect on lowering serum cholesterol and triglyceride, while increasing HDL cholesterol. Recently, Zhao et al. (2015) conducted the experiment to investigate the effect of dietary supplementation of 1.0 g/kg LPL in reduced ME at 10.57 and 10.32 MJ/kg during phase I and II for weaning pigs, respectively. The results demonstrated that pigs fed reduced energy diets with LPL had greater average daily gain (ADG) than weaning pigs fed standard energy level, and decreased triglyceride concentration. The positive effects on growth performance could be the result of increased palatability with the inclusion of LPL or the effect to increase feed and energy intake via the improvement in nutrient digestibility of DM, GE, CP and crude fat. This implies that LPL is not only being a specific emulsification property of fat but also increasing an energy-yielding source in suboptimal energy diet.

3.4.4 Poultry Studies

Ruju et al. (2011) conducted the experiment to investigate the effect of phospholipid-rich byproduct from rice bran oil on broiler performance and fat digestibility. In experiment I, 250 broilers were given rice bran lysolecithin (RBL) at 0, 0.5, 2, 8, and 32 g/kg. The authors demonstrated that supplementary level of RBL from 2 to 32 g/kg diet could be used an extra energy-yielding ingredient in young chickens in respect to increased fat digestibility. The higher digestibility of

fat with RBL supplementation was probably responsible for increased relative weight of pancreas which was important organ for pancreatic enzyme secretion. In experiment II, the authors found that feed conversion efficiency improved with 50 g RBL/kg diet compared to birds fed diets containing 0 and 25 g RBL/kg diet. It also observed that RBL inclusion in broilers' diets had beneficial effects on body weight, fat digestibility, and reduced liver weight in young chickens. The decrease in relative weight of liver in avian species is considered to be affected the de novo fatty acid synthesis which may alter metabolic pathway of fat and in turn modification of lipid metabolites (Ristic et al., 2005). Huang et al. (2008) proved this hypothesis by inclusion levels of 0, 50, and 10 g/kg soy-lecithin in corn-soybean basal diets for broilers. Results revealed that soy-lecithin influenced circulating concentrations of lipid metabolites, hepatic lipogenic hormone, hepatic genes, especially those genes regulating lipogenesis (acetyl-CoA carboxylase, malic enzyme, fatty acid synthase, and stearoyl-CoA desaturase). The increasing levels of lipogenic enzymes are directly effects glucose metabolism, fatty acid and lipid production and this expression is regulated by insulin (Ferré and Foufelle, 2010).

In laying hen studies, An et al. (1997) observed that addition of dietary safflower phospholipids (crude safflower and purified phosphospholipids) at 50 g/kg diet had higher egg production and daily egg mass than those fed tallow or the blend of safflower and palm oils. It also found the reductions of total cholesterol and free fatty acid concentration when hens fed crude safflower phospholipid, which was showed potential effect than those from purified source. Several investigations conducted in poultry trials have reported the effect of dietary lecithin on blood associated with lipid metabolism (An et al., 1997; Huang et al., 2008). Hypocholesterolemic effects with phospholipid may be attributed to the decreased cholesterol secretion or increased HDL uptake into the liver. These will be valuable feed additive to poultry studies for improving productivity and reducing

cholesterol content without any adverse effects in their body, which will be benefit for poultry producers and consumers preferences.

A potential effect of different phospholipid sources also investigated. A potential effect of different phospholipid sources also investigated. According to Han et al. (2010), who evaluated feeding level of lysolecithin at 0, 5, 10, and 15 g/kg diet for laying hens during 6 week-feeding period. The results found linearly improvement in egg weight, relative albumen and yolk weight, as well as fat soluble vitamins content in egg yolk. However, the inclusion of LPL in an isocaloric diet for mature layers was not decreased as the inclusion level increased, which was possibly affected by excess intake of energy. This result was contrast to Attia et al. (2009), who observed the effect of lecithin inclusion at 30 and 60 g/kg diet as a part of isocaloric and extra energy source in dual-purpose crossbred hens (Gimmizah and Silver Montazah strains) had no effects on metabolic profile of lipid either in the blood or the egg yolk. Although feeding lecithin to crossbred hens positively affected on laying performance, feed efficiency, fat digestibility, and reproductive performance, but there was not found hepatic alterations of key metabolic enzymes and cholesterol concentration. The explanations for the differences among authors with respect to the effects of dietary lecithin on poultry production are not exactly understand but might depend on strain of bird, the composition of diet, inclusion level, emulsified source or fatty profiles of different lipid sources. For example, Mandalawi et al. (2015) reported that dietary lecithin could be replaced in animal fat when included at the ratios of 40:0, 20:20, and 0:40 g/kg. There were showed to increase egg weight (60.1. 60.7, and 61.8 g, respectively), egg mass production (56.8, 57.5, and 58.8 g/d, respectively), yolk pigmentation (9.2, 9.2, and 9.5, respectively), feed conversion ratio per kilogram of egg produced (2.07, 2.07, and 2.03, respectively), but no effect on nutrient retention.

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Chapter III: Effects of Lysophospholipid Supplementation to Lower Nutrient Diets on Growth Performance, Intestinal Morphology, and Blood Metabolites in Broiler Chickens

ABSTRACT: The purpose of this research was to investigate the effects of dietary lysophospholipid (LPL) supplementation to diets lower in metabolizable energy (ME), crude protein including selected amino acids on growth performance, intestinal morphology, blood metabolites, inflammatory response, and carcass traits in broiler chickens. A total of 300 one-day-old male chicks (Ross 308) were assigned to 5 treatments, with 6 replications of 10 birds each in a completely randomized design. The 5 treatments were: positive control (PC) without LPL supplementation and adequate in all nutrients, negative control (NC) without LPL, and reduced 150 kcal/kg of ME and reduced 5% of total crude protein including Lys, Met, Thr, and Trp in a calculated amount relative to the PC, NC + 0.05% LPL (LPL05), NC + 0.10% LPL (LPL10), and NC + 0.15% LPL (LPL15). Broilers fed with NC diet had poorer growth performance and lighter relative weight of breast muscle compared with PC diet. Moreover, the NC birds were more susceptible to inflammation via modulating the secretions of tumor necrosis factor alpha (P = 0.011), interleukin-1 (P = 0.036) and increasing crypt depth of the jejunum and duodenum. However, feeding LPL linearly improved growth performance, feed conversion ratio, ether extract, and crude protein retention. The LPL supplementation on low-energy and nitrogenous diets showed significant lowering uric acid (P = 0.001) concentration. Furthermore, the inclusion of LPL to the NC diet could alleviate inflammation with a tendency to decrease crypt depth of the duodenum (P = 0.074) and tumor necrosis factor alpha (P = 0.082) concentration. These improvements also influenced carcass composition of the relative weights of

pancreas, breast and leg muscle. Conversely, the LPL supplementation showed no significant effects on relative weights of immune organs, gizzard, and abdominal fat. Overall, LPL promotes growth performance, nutrient utilization, gut health, anti-inflammation, and muscle yields when applied to diets of broiler chickens with lower levels of energy, crude protein including selected amino acids.

Key words: Lysophospholipid; Lower Nutrient Diets, Broiler Chicken, Growth Performance, Intestinal Morphology

INTRODUCTION

Fast-growing broilers require energy and nitrogenous compounds for supporting growth and performance. However, there are still controversial issues on feeding young birds high amounts of nutrients (Widyaratne and Drew, 2011; Rochell et al., 2016). Emulsifiers play an important role in aiding micelle formation. Lysophospholipids (LPL) are natural surfactants of hydrolyzed soy lecithin, which are produced by phospholipase A_2 to cleave one hydrophobic fatty acid from phospholipids (Joshi et al., 2006). Thus it is more efficient than lecithin in emulsifying properties and the subsequent effect on fat hydrolysis. The LPL's higher hydrophilic–lipophilic balance values of 2 to 12, which is higher than bile and lecithin (Van Nieuwenhuyzen and Tomás, 2008), and lower critical micelle concentration (0.02 to 0.2 mM/L) make it more effective than modified lechitin. This indicates the ability to form smaller micelles in the guts of animals and cause larger surface areas of lipid droplets for pancreatic lipases to interact. LPL also alters protein channel formation in the membrane by increasing ion exchanges (Maingret et al., 2000). Change in deformation energy increased number and size of the membranous pores and consequently increased flux rate of macromolecules across the cell membrane (Kelkar and Chattopadhyay, 2007; Lundbaek et al., 2010). Both mechanisms induce the transport of nutrients, from small particles such as calcium ions to large components such as polysaccharides to be broken down for absorption, leading to higher nutrient bioavailability for promising broiler performance.

Besides the important role in increasing membrane permeability for better lymphatic absorption of lipophilic substance (Nagano et al., 2009), the derivative lecithins also act as immunostimulants by promoting the influx of monocytes and enhancing macrophages during the pathogenic invasion (Ousman and David, 2000). This was consistent with Lewis et al. (2016), who demonstrated the activation of

lipid-soluble phosphatidylcholine in modulating cell proliferation and interleukin-2 secretion, and consequently promoted cell-mediated immunity. Lecithin derivatives have also been shown to prevent cellular damage (Maingret et al., 2000; Skoura and Hla, 2009) and improve broiler performance through increased nutrient utilization (Raju et al., 2011; Jansen et al., 2015). Based on these findings, we hypothesized that LPL would be a good material for improving nutrient digestion and absorption as well as activating the immune system of broiler chickens. However, supplementation of new biosurfactant LPL is not well known in broiler chicken studies, especially when applied in diets lower in ME, CP, and amino acids (AA). Consequently, the aim of the current study was to investigate the effects of dietary LPL supplementation in diets low in ME, CP including selected AA on growth performance, nutrient digestibility, intestinal morphology, and blood profiles in broiler chickens.

MATERIALS AND METHODS

All procedures for bird care and handling were approved by the national research committee of Animal Ethics, Seoul National University (Gwanak, South Korea).

Experimental Design, Diets, and Management

A total of 300 one-day-old Ross 308 male chicks were obtained from a local hatchery (Busung Farms, South Korea). The chicks were individually weighed on an electronic scale (model PB 1501, Mettler, Toledo, OH) and assigned to pens with 10 chicks of 6 replications using a completely randomized design. The pen size was a 1.42 m x 1.31 m (1.86 m^2/pen) providing a stocking density of 5.38 birds/m^2 (10 birds/pen). Each pen was equipped with tube feeders, automatic waterers, and rice hull. The five treatments were: positive control (PC) formulated with adequate

amounts of ME, CP and AA with no supplementation of LPL, negative control (NC) without LPL supplementation, and reductions of 150 kcal of ME /kg and 5% of total CP including Lys, Met, Thr, and Trp in PC diet, NC + 0.05% LPL (LPL05), NC + 0.10% LPL (LPL10), and NC + 0.15% LPL (LPL15). The LPL derived from soy lecithin was obtained from Easy Bio Inc., (LIPIDOL, Seoul, South Korea). The content of LPL in this study was 37.5 g/kg product. The experimental diets during starter (d 0 to 7), grower (d 8 to 21), and finisher (d 22 to 35) periods were formulated according to the nutrient requirements for Ross 308 broilers (Aviagen, 2007; Table 1). Broilers were raised in a temperature-controlled environment for a 35 d feeding. The temperature was controlled at 34±2 ^{o}C for the first 3 days using a heat brooder, and gradually declined thereafter to 22 to 24 ^{o}C. During the course of the experiment, a lighting program offered a 23-h photoperiod (23L:1D) according to the management guidelines of commercial broilers. Drinking water and feed were provided *ad libitum* throughout the study.

Performance Measurements

Broiler BW and feed intake (FI) were recorded on pen basis at the d of hatch, 7, 21, and 35 d. The data were used to calculate body weight gain (BWG), FI, and feed conversion ratio (FCR) in each period and cumulatively. Mortality was recorded daily to adjust FI.

Nutrient Retention

The metabolic trial was conducted during 17 to 23 d of age. A total of 30 birds (one bird per pen), a mean BW of 678 g per bird were randomly chosen and placed individually into a cage (39 m x 50 m x 46.6 m). Plastic-coated pans were placed under each cage. The grower diet was mixed with ferric oxide (Fe_2O_3) at 10 g/kg of diet as initial and ended markers. Birds were fed experimental diets for 4 d

in an adjustment period (from 17 to 20 d of age). From d 21 to 23, total excreta of feces and urine were collected. Contaminations of scales, feather, and filoplumes were removed from the fecal samples every 12 h, then immediately stored frozen at -20 ^{o}C. All representative samples were pooled and dried in an oven using air-force drying for 72 h at 60 ^{o}C. Experimental diet and dried excreta analyses were performed using the standard protocols of AOAC International (2000) for measuring nutrient retention of CP by the Kjeldahl procedure (method 984.13), ether extract (EE) by the Soxhlet analysis (method 920.39), and DM (method 930.15). The analyzed values of ingested and excreted nutrients were used to calculate apparent total tract digestibility. Each sample was run in triplicate.

Intestinal Morphology

At the last day of experiment (d 35), 30 birds (one bird per pen) with the similar BW to the pen mean BW were randomly selected. Tissue samples of the duodenum and jejunum were collected and rinsed with buffered saline solution. An approximately 5 cm piece of the middle part of each segment was excised and fixed in 10% (vol/vol) saline solution for histomorphological measurements. Tissues from the segments preserved in 10% neutral formaldehyde solution were cut into 10 cross sections (approximately 3 μm thickness) and were fixed on slides for staining with hematoxylin and eosin. Villous height was defined from the villous tip to the villus to crypt junction, whereas crypt depth was measured from the villous bottom to the crypt. The ratio of villous height and crypt depth (VH:CD ratio) was examined. Morphological studies were performed on a light microscope (Olympus Corporation, Tokyo, Japan) with stereological software analysis using Version 2.3.1.3 (Visiopharm Albertslund, Hørsholm, Denmark).

Blood Collection and Analyses

Blood was randomly collected from jugular veins after a 2-hr feeding withdrawal from 6 birds at the beginning of the experiments and 30 birds at the end of the experiments (one sample from each replication). The samples were immediately transferred into non-heparinized vacuum tubes, placed at room temperature for 2 h for serum separation, and thereafter centrifuged (3,000 x g) at 4 ^{o}C for 10 min. The serum without supernatant was removed into vials and immediately delivered to the laboratory for further biochemical analyses. Serum interleukin-1 (IL-1, catalog no. CSB-E10069Ch), interleukin-6 (IL-6, catalog no. CSB-E08549Ch), and tumor necrosis factor alpha (TNF-α, catalog no. CSB-E11231Ch) were analyzed using commercial ELISA kits (Cusabio Biotech Inc., Wuhan, China). Circulating serum free fatty acid (FFA, catalog no. 438-91691) was determined using commercial kits (Wako Pure Chemical Industries, Osaka, Japan). Uric acid (catalog no. P803-OU982-01) was quantified using commercial reagent kits (Pointe Scientific, Canton, MI). All representative samples were performed twice and measured immediately to avoid variations.

Carcass and Intestinal Organ Measurements

After collecting the blood, the selected birds were sacrificed by cervical dislocation for carcass measurements. Carcass components of immune organs (thymus, spleen, and bursa of Fabricius), gizzard, pancreas, breast, leg muscle, and abdominal fat were weighed on an electronic scale for further calculation of relative carcass weights.

Statistical Analysis

Data were analyzed in a completely randomized design using the GLM procedure of SAS software (SAS Institute, Cary, NC). The pen was an experimental unit for growth performance, whereas all selected birds from each replication were

defined as the experimental unit for nutrient retention, blood metabolite, intestinal morphological, and carcass measurements. T-test comparison was used to separate significant differences between the control treatments. All criteria were assessed for linear and quadratic effects of LPL supplementation by orthogonal polynomial contrast. The significant difference for a tendency was defined at $P > 0.05$ to $P < 0.10$.

RESULTS

Growth Performance

As shown in Table 2, growth performance was not affected by the dietary inclusion of control diets in a starter period. The supplementation of PC had higher BW ($P = 0.040$), BWG ($P = 0.048$), and had lower FCR ($P = 0.048$) during 8 to 21 day of age. The average BWG and FI were numerically higher and the average FCR was lower for broilers fed with PC compared with the NC birds in the finisher and the whole experiments ($P = 0.001$). However, the increasing level of LPL in the NC diet could minimize these detrimental effects through an increase in bird BW during the grower period ($P = 0.038$), and both linear and quadratic effects during the finisher period ($P < 0.001$). The average BWG tended to improve from 789.35 to 840.51 g/bird in the growing period as the inclusion level of LPL increased ($P = 0.060$). The linear and quadratic improvements for BWG with the supplementation of LPL were also observed in the finishing and overall period ($P < 0.001$). Moreover, increased level of LPL could stimulate the FI (linear and quadratic effects; $P = 0.001$) and decrease FCR (linear and quadratic effects; $P = 0.003$ and $P = 0.007$, respectively) of the birds from 22 to 35 day of age and 0 to 35 day of age.

Nutrient Retention

Nutrient retention for DM, CP, and EE are summarized in Table 3. Birds in

control treatments had no considerable differences in nutrient retention of DM, CP, and EE. However, the linear and quadratic effects for nutrient retention of CP (P = 0.020 and P = 0.014, respectively) were detected as the LPL level increased. Furthermore, supplementation of LPL showed an improvement in EE retention in a linear response (P = 0.025).

Intestinal Morphology

The effects of LPL supplementation on gut morphology is summarized in Table 4. There were no significant differences in villus height, jejunal crypt depth, and jejunal VH:CD between broilers fed PC and NC diets. Longer crypt depth (P = 0.017) and shorter VH:CD (P = 0.090) in the duodenal segment were found in the NC birds compared to those in PC. No linear and quadratic effects were observed with LPL supplementation in all criteria of intestinal morphology. However, inclusion of LPL had a tendency effect on diminished crypt depth in the duodenum (P = 0.074) when increasing the LPL level.

Blood Metabolic Profiles

Blood metabolites of inflammatory response at the initial and the terminal periods are indicated in Table 5. No significant differences were observed between the control groups with respect to IL-6 and uric acid concentrations. However, the NC birds were more susceptible to increase inflammation by increasing serum concentrations of TNF-α (P = 0.001) and IL-1 (P = 0.036) than those in PC treatment. In addition, higher level of FFA was found in the NC compared to the PC treatment (P = 0.048). The LPL supplementation to lower nutrient diets contributed to a tendency decrease (P = 0.082) in the TNF-α concentration and to a significant decrease (P = 0.001) in uric acid concentration in response to increased LPL levels. However, metabolic profiles of IL-1, IL-6, and FFA concentration

were unaffected by increasing the LPL level.

Relative Weights of Immune Organs and Carcass Components

Table 6 shows the effects of LPL supplementation to lower nutrient diets on relative weights of immune organs and carcass weights in broiler chickens. The standard nutrient diet of PC treatment did not have superior increases in the relative weights of immune organs and carcass weights of gizzard, pancreas, leg muscle, and abdominal fat pad compared with the lower nutrient diet of NC treatment. However, the PC-supplemented diet produced a greater weight of breast muscle compared to those of the NC-supplemented diet ($P = 0.020$). The LPL showed the linear ($P = 0.002$) and quadratic ($P = 0.005$) improvements in the relative weight of breast muscle. In addition, there were significantly increased in the relative weights of pancreas (a linear effect; $P = 0.035$) and leg muscle (a quadratic effect; $P = 0.044$) as the inclusion rate of LPL increased. No significant differences were detected in the relative weights of thymus, spleen, Bursa, gizzard, and abdominal fat by the LPL supplementation.

DISCUSSION

Growth Performance

The use of LPL is important for commercial broilers under the limitations of energy and nitrogenous diets. The current research found LPL supplementation beneficial for adequate nutrient supply as well as activation of various functions in the body. Some studies showed improvement in body weight gain (Emmert et al., 1996) and G:F ratio (Khonyoung et al., 2015) of young birds fed emulsifiers. The previous results are in agreement with our findings, regardless of BW and BWG. This supports the important function of phospholipids in fat digestion by their emulsifying properties and nutrient absorption via increasing micelle formations

(Schwarzer and Adams, 1996), resulting in better growth performance in young chicks. The decrease in FI of the NC birds was contrast to previous report (Ferreira et al., 2016), in which birds showed to consume more feed under the restriction of nutrient in order to meet their nutrient requirements. The different finding found in the current study might influence by pathological status of the birds though the modulating secretions of inflammatory cytokines.

Nutrient Retention

In low nutrient diets, LPL has been proven to increase the nutrient retention and energy values in feedstuffs. According to Zhao et al. (2015), who observed that weaning pigs fed a restricted energy diet at 0.30 MJ/kg in early and late weaning periods with the inclusion level of LPL at 0.05% had greater digestibility of DM, gross energy, CP, and EE than the basal and reduced nutrient diets without LPL supplementation, which was in accordance with the current findings of greater CP and EE retention. Similarly to Jansen et al. (2015), who found that the digestibility of fatty acids, EE, and AME_n values were improved by feeding phospholipids to broiler chickens. These improvements agreed with the observation of Han et al. (2010), who showed that addition of lysolecithin at 0.10% in laying hen diets could maximize digestibility of nitrogen, energy, and AA. Modified lecithins are known to be an integral part of phospholipid bilayers (Shumilina et al., 2006). It acts as an important regulator in modifying fluidity and permeability of lipid bilayer by decreased deformation energy, which directly affects the stability of the cell membrane. This means that the coupling between integral membrane proteins and their surrounding lipid bilayers will alter the hydrophobic interface to enter protein channel (Lundbaek et al., 2010). This process causes increased flux rate of various nutrients as well as promoted absorption of lipid and lipophilic substances for entering the enterocyte (Cohn et al., 2010). Once

it is taken up by the enterocyte, the LPL is converted to phospholipids and the delivery of absorbed lipids is further assisted by chylomicrons (Nakano et al., 2009), resulting in sufficient nutrients to support growth and meat yield.

Small Intestinal Morphology

The morphology of intestinal mucosa is one of indicative biomarkers to determine gut health. Changes in intestinal mucosa such as decreased villi length or increased crypt depth have been considered to cause tissue damage induced by invading pathogens (Nabuurs et al., 1993). Our results demonstrated that the longer crypt depth of the duodenum was significantly increased in the NC treatment, indicating a higher rate of epithelial cell turnover in response to inflammation or pathogen invasion to renew the damaged tissue. Furthermore, the lower VH:CD ratio in broiler fed the NC diet resulted in an increase tissue turnover of the duodenal mucosa. A higher turnover rate has significant influence on a higher maintenance requirement, which can finally lead to retarding broiler performance (Khongyoung et al., 2015). However, the LPL showed to decrease a lower rate of cellular turnover via a shortage of crypt depth in the jejunum. The diminished crypt depth has been pronounced in lowering the rate of epithelial cell destruction, inflammation, and sloughing of the intestinal segment from bacterial infections (Yason et al., 1987). In addition, the mature apical enterocyte of the LPL-broilers could control the enterocyte migration, and normal sloughing (Khongyoung et al., 2015). These findings were considered to improve broiler performance, nutrient utilization, and anti-inflammation. Therefore, the enhanced development of intestinal mucosa may have primary influences on changes of gut morphology, and defensive mechanism and subsequent effect on bird performance and immunity.

Inflammatory Response and Metabolic Function

Birds in the NC were more susceptible to increase TNF-α and IL-1, which are acute phase proteins in activating inflammation. Because the primary function of TNF-α is induced IL-1 and IL-6 secretions (Nakano et al., 2009), resulted in poorer broiler performance and feed efficiency in the NC birds. However, there are numerous studies have indicated that biosurfactant phospholipids play an important role in immunological process (Hartmann et al., 2009). Our study also agreed with previous works on a potential lowering effect in the secretion of acute phase protein of TNF-α concentration, implicating that the production of proinflammatory cytokines is inhibited by increasing hemolytic phagocytosis (Zhao et al., 2011). The key roles of phospholipids in regulating inflammation and innate immunity of cytokines and chemokines were observed by Yun et al. (2005). In vitro study also observed that the LPL can directly integrate in the mucus layer as well as in the membrane of an enterocyte by blocking proinflammatory signals in Caco-2 cells (Nakano et al., 2009). This study showed that it may be possible for LPL to change the barrier properties of the mucosa, therefore alleviating inflammatory response to pathogenic invasion, which is in accordance with morphology criteria.

The FFA concentration was greater for birds fed NC than for those fed PC. It might be affected by modulating the effects of diacylglycerol and triacylglycerol synthesis, which can be hydrolyzed to release FFA (Jansen et al., 2015), so that fat mobilization may increase in response to the demand of energy. Serum uric acid concentration was also in NC treatment. It is known as a major end product of N metabolism in broilers, and thus decreased uric acid concentration is considerable to determine AA utilization (Donsbough et al., 2010). The lowering N excretion might be associated with decreased crude protein level as suggested by Han et al. (2010). The inclusion of LPL also had a positive effect on lowering uric acid secretion. The LPL are well established to alter the phospholipid bilayer of cell membranes, which

allow the uptake of nutrients across the enterocyte (Lundbaek et al., 2010). This is confirmed by the improvements in metabolic profiles of proteins, carbohydrate and lipid similar to previous research (Huang et al., 2008; Han et al., 2010; Zhao et al., 2015). The function of modified lecithin in lowering N excretion might be associated with improved CP retention, similarly to the finding of Han et al. (2010). It is indicated that low nitrogenous diets with LPL supplementation are sufficient for maximizing amino acid utilization. This is consistent with the longer villus height and the greater CP retention in LPL-supplemented treatments.

Relative Weights of Immune Organs and Carcass Composition

Dietary supplementation of LPL had no significant effect on the relative weight of lymphoid organs, whereas it improved the relative weights of breast and leg muscles. These findings were inconsistent with Cho et al. (2012), who found that inclusion of 0.05% sodium steroyl-2-lactylate could increase spleen weight but had less impact on carcass yield when added to diets of 150 kcal/kg less than the commercial recommendation. The emulsifier source and dietary composition may affect carcass inconsistency results. The current study also found an increase in pancreas size of LPL-supplemented diet, which was in agreement with Raju et al. (2011), who observed greater pancreas weight in broilers fed 0.5 g/kg lysolecithin. The increase of relative pancreas weight may promote the hydrolysis of triglycerol for greater digestion and absorption of lipids. The significant lowering effect of abdominal fat was not observed in this study. It is possible that LPL alters the facilitation of lipid and protein fractions in the circulation for conversion into the muscles rather than abdominal fat deposition, which consequently affects fatty acid and amino acid deposits in the meat.

CONCLUSION

Supplementation with LPL on low-nutrient diets improved broiler performance, nutrient digestibility, intestinal morphology, carcass yields, metabolic profiles, and lowered inflammation. Additionally, the growth performance of birds fed with NC diet supplemented with LPL appeared to be higher than NC.

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Table 1. Composition and nutrient specifications for the experimental diets (%, as fed basis)

	Starter (d 0-7)		Grower (d 8-21)		Finisher (d 22-35)	
Item	PC	NC	PC	NC	PC	NC
Fine corn	40.81	44.61	28.16	30.60	15.27	16.40
Coarse corn	13.60	14.87	28.15	30.60	45.82	49.22
Soybean meal	30.98	27.93	27.62	24.68	22.81	20.13
DDGS	3.00	3.00	3.00	3.00	3.00	3.00
Meat meal	3.00	3.00	4.00	4.00	4.00	4.00
Soybean oil	3.73	1.00	5.20	2.50	5.44	2.80
L-Lysine sulfate (55%)	0.45	0.47	0.25	0.28	0.23	0.25
DL-Methionine (98%)	0.37	0.34	0.29	0.27	0.25	0.23
Threonine (98%)	0.13	0.13	0.07	0.06	0.05	0.06
Tryptophan (10%)	0.03	0.07	0.00	0.04	0.00	0.04
Choline	0.08	0.08	0.07	0.07	0.08	0.08
Dicalcium phosphate	2.10	2.10	1.70	1.70	1.60	1.60
Limestone	1.11	1.79	0.89	1.60	0.85	1.59
Dried salt	0.30	0.30	0.30	0.30	0.30	0.30
$NaHCO_3$	0.06	0.06	0.05	0.05	0.05	0.05
Vitamin premix[1]	0.13	0.13	0.13	0.13	0.13	0.13
Mineral premix[2]	0.12	0.12	0.12	0.12	0.12	0.12
Calculated value[3]						
ME (kcal/kg)	3,025	2,875	3,150	3,000	3,200	3,050
CP (%)	22.00	20.90	21.00	19.95	19.00	18.05
Ether extract (%)	6.73	3.99	8.19	5.61	8.50	5.96

Lysine (%)	1.40	1.34	1.23	1.17	1.09	1.03
Methionine (%)	0.69	0.65	0.60	0.57	0.54	0.51
Threonine (%)	0.94	0.90	0.84	0.80	0.75	0.72
Tryptophan (%)	0.25	0.23	0.23	0.22	0.20	0.19

PC = positive control, NC = negative control, DDGS = distiller's dried grains with solubles.

[1] Provided the following quantities of vitamin mixture per kilogram of complete diet: vitamin A (retinyl acetate), 11,000 IU; vitamin D_3, 5,000 IU; vitamin E (dl-α-tocopheryl acetate), 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; and calcium pantothenic acid, 20 mg.

[2] Provided the following quantities of mineral mixture per kilogram of complete diet: Cu (copper sulfate), 29 mg; Zn (zinc sulfate), 108 mg; Mn (manganese sulfate), 115 mg; Fe (ferrous sulfate), 60 mg; and Se (sodium selenite), 0.4 mg.

[3] Calculated values.

Table 2. Effects of LPL supplementation to lower nutrient diets on growth performance in broiler chickens[1,2]

				P - value					*P* – value	
Criteria	PC	NC	SEM[3]	PC vs. NC	LPL05	LPL10	LPL15	SEM[3]	Linear	Quadratic
Body weight (g/bird)										
1 wk	182.45	180.32	5.459	0.856	179.63	182.53	182.49	2.980	0.618	0.932
3 wk	1,038.83	969.67	17.453	0.040	977.45	1,018.26	1,023.00	10.651	0.038	0.755
5 wk	2,240.37	1,649.00	94.563	<0.001	2,123.07	2,121.46	2,173.63	44.153	<0.001	<0.001
BW gain (g/bird)										
0 to 1 wk	140.42	138.29	5.459	0.856	137.60	140.50	140.46	2.980	0.618	0.932
2 to 3 wk	856.38	789.35	17.443	0.048	797.81	835.74	840.51	10.357	0.066	0.763
4 to 5 wk	1,201.54	679.33	83.532	<0.001	1,145.63	1,103.21	1,150.63	39.745	<0.001	<0.001
0 to 5 wk	2,198.34	1,606.97	108.384	<0.001	2,081.04	2,079.43	2,131.60	44.153	<0.001	<0.001
Feed intake (g/bird)										
0 to 1 wk	152	151	4.201	0.886	152	152	152	2.523	0.812	0.868
2 to 3 wk	1,198	1,162	21.619	0.434	1,169	1,185	1,192	11.934	0.391	0.799

				P - value					*P* – value	
Criteria	PC	NC	SEM[3]	PC vs. NC	LPL05	LPL10	LPL15	SEM[3]	Linear	Quadratic
4 to 5 wk	1,951	1,395	94.877	0.001	2,001	1,967	2,011	51.435	<0.001	0.001
0 to 5 wk	3,301	2,708	94.563	0.001	3,322	3,304	3,355	56.033	<0.001	0.001
Feed conversion ratio (feed:gain)										
0 to 1 wk	1.09	1.11	0.035	0.759	1.11	1.09	1.08	0.015	0.589	0.912
2 to 3 wk	1.40	1.47	0.020	0.048	1.47	1.42	1.42	0.015	0.208	0.946
4 to 5 wk	1.63	2.06	0.084	0.003	1.75	1.79	1.75	0.035	0.003	0.007
0 to 5 wk	1.50	1.68	0.035	0.003	1.60	1.59	1.58	0.015	0.012	0.083

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL.

[1] A total of 300 chickens were fed diets from the initial BW of 42.03 g/bird to the final BW of 2,061.51 g/bird.

[2] Each value represents least squares mean of 6 replications of 10 broiler chickens.

[3] SEM = standard error of the means.

Table 3. Effects of LPL supplementation to lower nutrient diets on nutrient retention during 21 to 23 d of age[1]

				P - value					*P* – value	
Criteria	PC	NC	SEM[2]	PC vs. NC	LPL05	LPL10	LPL15	SEM[2]	Linear	Quadratic
Nutrient retention (%)										
DM	63.63	64.04	1.933	0.921	64.61	63.46	64.97	1.446	0.916	0.765
CP	68.40	66.01	1.683	0.504	74.82	74.93	74.63	1.148	0.002	0.014
EE	67.36	64.54	3.255	0.686	80.10	79.93	80.03	2.194	0.025	0.079

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL

[1] Values are expressed as means of 6 birds represented from each treatment (one bird per replication, n = 30).

[2] Standard error of the means.

Table 4. Effects of LPL supplementation to lower nutrient diets on intestinal morphology in broiler chickens at 35 d of age[1]

				P - value					*P* - value	
Criteria	PC	NC	SEM[2]	PC vs. NC	LPL05	LPL10	LPL15	SEM[2]	Linear	Quadratic
Villus height (μm)										
Jejunum	981.42	978.73	57.302	0.983	1,076.95	1,171.29	1,092.90	33.464	0.153	0.919
Duodenum	847.56	917.28	53.496	0.541	1,076.95	1,025.83	1,045.36	34.474	0.315	0.245
Crypt depth (μm)										
Jejunum	193.15	203.51	6.078	0.420	166.29	190.64	193.94	4.037	0.913	0.074
Duodenum	121.81	158.01	8.113	0.017	133.16	161.54	170.41	4.761	0.115	0.376
Villus height : crypt depth										
Jejunum	5.10	5.05	0.454	0.952	6.57	6.20	5.65	0.246	0.554	0.374
Duodenum	7.02	5.86	0.402	0.159	8.13	6.41	6.25	0.276	0.837	0.090

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL.

[1] Values are expressed as means of 6 birds represented from each treatment (one bird per replication, n = 30).

[2] Standard error of the means.

Table 5. Effects of LPL supplementation to lower nutrient diets on blood profiles in broiler chickens[1,2]

				P - value					*P* – value	
Criteria	PC	NC	SEM[3]	PC vs. NC	LPL05	LPL10	LPL15	SEM[3]	Linear	Quadratic
TNF-α (pg/mL)										
Initial[2]	6,819.59	6,819.59	-	-	6,819.59	6,819.59	6,819.59	-	-	-
d 35	2,608.55	2,998.58	83.579	0.011	2,742.57	2,630.98	2,643.43	56.772	0.082	0.402
IL-1 (pg/mL)										
Initial[2]	142.18	142.18	-	-	142.18	142.18	142.18	-	-	-
d 35	65.48	90.09	6.092	0.036	80.12	76.67	76.56	3.019	0.117	0.408
IL-6 (pg/mL)										
Initial[2]	10.17	10.17	-	-	10.17	10.17	10.17	-	-	-
d 35	8.08	9.14	0.332	0.112	8.94	7.91	8.94	0.234	0.513	0.457
Free fatty acids (mg/dL)										
Initial[2]	151.37	151.37	-	-	151.37	151.37	151.37	-	-	-
d 35	326.50	541.33	55.761	0.048	549.83	496.33	482.17	28.979	0.411	0.964

Uric acid (mg/dL)										
Initial[2]	4.80	4.80	-	-	4.80	4.80	4.80	-	-	-
d 35	4.78	4.60	0.244	0.726	4.35	3.12	3.12	0.196	0.001	0.668

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL, TNF-α = Tumor necrosis factor alpha; IL-1 = Interleukin-1; IL-6 = Interleukin-6.

[1] Values are expressed as means of six birds represented from each treatment (one bird per replication, n = 30).

[2] Initial value is represented as mean of six birds collected samples at one d of age (n = 6).

[3] Standard error of the means.

Table 6. Effects of LPL supplementation to lower nutrient diets on relative immune organs and carcass weights in broiler chickens at 35 d of age[1,2]

				P - value					*P* – value	
Criteria	PC	NC	SEM[3]	PC vs. NC	LPL05	LPL10	LPL15	SEM[3]	Linear	Quadratic
Immune organs (g/100 g BW)										
Thymus	0.46	0.44	0.026	0.695	0.47	0.45	0.44	0.014	0.934	0.786
Spleen	0.09	0.09	0.008	0.922	0.07	0.08	0.07	0.005	0.159	0.244
Bursa	0.23	0.21	0.009	0.359	0.24	0.22	0.21	0.006	0.710	0.410
Carcass traits (g/100 g BW)										
Gizzard	2.24	2.24	0.130	0.995	2.26	2.25	2.25	0.057	0.951	0.950
Pancreas	0.27	0.23	0.011	0.175	0.29	0.29	0.29	0.008	0.035	0.119
Breast	18.63	15.71	0.668	0.020	18.96	18.36	20.19	0.405	0.002	0.005
Leg	18.12	17.50	0.249	0.230	18.79	18.36	18.48	0.157	0.100	0.044
Abdominal fat	1.13	1.21	0.088	0.664	1.17	1.19	1.18	0.064	0.936	0.859

PC = positive control, NC = negative control, LPL05 = NC + 0.05% LPL, LPL10 = NC + 0.10% LPL, LPL15 = NC + 0.15% LPL.

[1] Values are expressed as means of six birds represented from each treatment (one bird per replication, n = 30).

[2] Initial value is represented as mean of six birds collected samples at one d of age (n = 6).

[3] Standard error of the means.

Chapter IV: Effects of Dietary Lysophospholipid Supplementation on Egg Production, Lipid Metabolism, and Fatty Acid Deposition in Brown Egg-Laying Hens

ABSTRACT: This study examined the effects of lysophospholipid (LPL) supplementation on egg production, egg quality, lipid metabolism, and fatty acid deposition of brown egg-laying hens from 28 to 38 wks of age. A total of 420 Hy-Line W36 laying hens were allotted into five dietary treatments with six replicates and 14 hens in each treatment based on a completely randomized design. Hens were fed 0 (CON), 0.025 (LPL25), 0.05 (LPL50), 0.075 (LPL75), and 0.10% (LPL100) LPL in the five dietary treatments. There were no significant differences in laying performance and egg quality among treatments. However, increasing LPL level showed linear effects on hen-day production ($P = 0.009$) and egg mass ($P = 0.005$). The hens fed LPL75 was greater hen-day production ($P < 0.05$), egg mass ($P < 0.01$), and yolk color ($P < 0.05$) than those fed CON. Linear increase in dark yolk pigmentation was also observed for laying hens fed dietary LPL ($P = 0.038$). No significant differences were observed in the nutrient retention of dry matter, crude protein and ash. However, crude fat retention tended to improve with increasing levels of LPL ($P = 0.051$). Cholesterol fractions, vitamins A and E concentrations were unaffected by dietary treatments at 33-wk of age. However, linear effects were observed for triglyceride ($P = 0.020$) and vitamin A ($P = 0.022$) concentrations at 38 wks of age. The LPL100 lowered cholesterol in the blood compared to CON. Furthermore, diets containing LPL100 had large percentage of C18:2n-6 and large ratio of polyunsaturated fatty acids to saturated fatty acids (SFA) than CON ($P < 0.05$). The supplementation of LPL also decreased the deposition percentage of SFA (linear effects; $P = 0.003$). Overall, the LPL can be used in laying hen diets to reduce cholesterol fractions and increase laying performance.

Key words: Lysophospholipid, Laying Hen, Productive Performance, Egg Quality Fatty Acids

INTRODUCTION

Increasing consumer demands for functional foods based on animal products, such as chicken meat and eggs, are emerging as health issues. Eggs are well established as excellent sources of essential nutrients for human health and neonatal growth (Simopoulos and Salem, 1992). However, the daily consumption of eggs should be limited due to high levels of cholesterol (approximately 275 mg per egg) and saturated fatty acids (SFA), which are harmful to human health, especially in patients with cardiovascular diseases (Spence et al., 2010). The egg industry should focus on dietary manipulations to modify the proportion of yolk lipids to adjust cholesterol levels and fatty acid profiles. These modifications should decrease the negative impacts of high cholesterol intake. Several approaches have been suggested to lower cholesterol contents in yolk (Chowdhury et al., 2005; Pekel and Alp, 2011).

Recently, feeding strategies to increase natural phospholipids, such as lecithin, lysolecithin, and lysophospholipid, have been intensively studied in swine (Zhao et al., 2015) and poultry (Attia et al., 2009). Previous studies indicated potential effects of phospholipids on broiler performance (Raju et al., 2011), intestinal mucosa (Khonyoung et al., 2015), and nutrient digestibility (Zhang et al., 2011). The function of emulsifiers on productive performance in laying hens was recently reported by Mandalawi et al. (2015), who demonstrated that lecithin could be substituted with animal fat as an energy source with beneficial effects on egg weight, egg mass and feed conversion ratio. Han et al. (2010) also showed a linear increase in egg weight when supplementing lysolecithin up to 0.15% and observed better assimilation of fat-soluble vitamins into the yolk. According to Attia et al. (2009), the addition of 6% lecithin in dual-purpose crossbreed hens significantly increased yolk percentage, Haught unit and yolk color. Additionally, many studies

observed advantageous effects of lecithin on hypocholesterolemic properties in animals (Hunt and Duncan, 1985; Huang et al., 2008) and humans (Wójcicki et al., 1995). Jansen et al. (2015) showed enriched sources of ω-6 fatty acids (50.61%) and polyunsaturated fatty acids (PUFA, 65.22%) and low amounts of SFA (23.93%) in soybean lecithin. However, available data on the effects of dietary lysophospholipid (LPL) for decreasing cholesterol and triglyceride contents without affecting fatty acid profile deposition in egg yolk is scarce. We hypothesized that high amounts of PUFAs and ω-6 fatty acids may be better incorporated in micelles by LPL and transported into yolk; this may be favorable for consumer health. Consequently, the objectives of this study were to examine the effects of various LPL levels of 0.025, 0.05, 0.075 and 0.10% on 1) egg production and interior egg quality from 28 to 38 wks of age, 2) apparent nutrient digestibility after a 5-wk feeding period, 3) blood metabolites related to lipid metabolism, and 4) fatty acids profiles in egg yolk at 38 wks of age in laying hens.

MATERIALS AND METHODS

The guideline of bird handling and management in this research were approved by the Animal Ethics Committee of Seoul National University (Gwanak, South Korea).

Experimental Birds, Dietary Treatments and Management

A total of 420 Hy-Line W36 laying hens (28 wks of age) with an average hen-day egg production percentage of 86.73±1.83 were allotted into five dietary treatments of six replicates each. Each treatment consisted of 14 hens, and two layer hens were assigned per cage. All selected birds were subjected to the five treatments based on a completely randomized design. The dietary treatments were supplemented with 0 (CON), 0.025 (LPL25), 0.05 (LPL50), 0.075 (LPL75) and

0.10% LPL (LPL100) (Easy Bio, Inc., Seoul, South Korea). The Lipidol^{TM} product contains 37.5 g/kg LPL. The laying hens were housed in 39 m x 50 m x 46.6 m (width x height x length) wire-floored, automatically ventilated cages for 10 week-feeding periods. The birds were raised in temperature-controlled houses under non-natural light for a 16-h photoperiod (16L:8D) throughout the entire experiment. All birds were provided access to feed and drinking water ad libitum. The formulation used in this study met or exceeded requirements for Hy-Line W36 brown laying hens (Hy-Line International, 2006-2008). The ingredients and nutrient compositions of the experimental diets are listed in Table 1.

Egg Production and Egg quality Measurements

Hens were weighed at the beginning (wk 28) and at the end of the study (wk 38). The number of eggs produced in each replicate was recorded daily at 1500 h and expressed as percentages of hen-day egg production. Feed intake (FI) was measured every 14 days and reported cumulatively. Egg weights and numbers of broken and abnormal eggs were recorded daily. From these data, egg weights, egg masses, feed intake, feed conversion ratio (FCR), feed per dozen eggs, and cracked and abnormal eggs (smaller eggs, soft-shell eggs, slab-sided eggs, yolkless eggs, wrinkled eggs and misshapen eggs) were cumulatively calculated.

A total of 36 fresh eggs (n = 180) with an average egg weight of 62.15±2.38 from each treatment were selected on the last day of a 14-day interval period to measure egg quality. All eggs were compressed using an IMDA digital force reader gauge (model MTG-40, Fugihira Industry Co., Ltd., Tokyo, Japan) to determine the eggshell breaking strength, which was detected from the peak point of each loading curve. The blunt edge of each egg was used to determine the eggshell color with a QCM (TSS, York, UK). Interior egg quality properties, such as albumen height and yolk color, were determined after breaking the egg using a

Multitester equipment (QCM System, Technical Services and Supplies, Dunnington, York, UK). The albumen height and egg weight were automatically calculated and reported as the Haugh unit. Eggshell thickness was measured after washing and drying eggs at room temperature for 74 h. Each sample was measured at 3 different positions on the egg (at the middle, at the sharp end and at the blunt end) using a digital micrometer to ±0.001 mm accuracy (model IT-014UT, Mitotuyo Co., Ltd., Kawasaki, Japan). The average of the three measurements was reported as the eggshell thickness. The percentages of eggshell and yolk were determined thereafter by weighing each part of the egg on a digital balance (model PB 1501, Mettler, Toledo, OH) and reported as percent weights of egg compositions divided by egg weight x 100. Albumen weight was determined by subtracting the eggshell plus egg-yolk weight of the overall egg weight. All measurements of egg quality were cumulatively summarized.

Nutrient Retention Assays

A total of seven laying hens from each group were randomly chosen and placed in a metabolic cage for the determination of nutrient retention at 33 wks of age. Each cage was equipped with feeders, waterers and collection trays. Ferric oxide (Fe_2O_3, 10 g/kg diet) was supplemented in the diet as an indigestible marker at the initial and end of fecal collection. The trial was conducted for seven days with a four-day adjustment period and three days for excreta collection. During the three days collection period, total excreta without scales, filoplumes and feathers were collected two times daily (at 12-h intervals), weighed and kept in sealed plastic bags. Pooled samples from each cage were air-dried at 60 °C for 72 h to constant weights and were then ground into small 1.0-mm-sized particles using a hammer mill (model Z-I, Retsch, Stuttgart, Germany) for DM (procedure 930.15), CP (procedure 984.13), crude fat (procedure 920.39) and total ash (procedure

942.05) analyses using the standard protocols of AOAC International (2000). Each sample of excreta and diet were assayed in triplicate. The percentage of nutrient retention was calculated according to formulas described by Zhang et al. (2011).

Blood Collection and Analyses

Laying hens (89% average egg production) were randomly selected to collect blood from the jugular vein at 33 and 38 wks of age from 30 laying hens. Five milliliters of blood were taken from a left wing vein with sterilized needles and syringes and performed within a minute to diminish handling stress. The samples were directly transferred into anticoagulant tubes, stored at 25 °C for 2 h, and centrifuged at 3,000 rpm at 4 °C for 15 min. The collected sera were stored at -80 °C for subsequent analyses. All metabolic profile measurements were analyzed using commercial kit tests (Roche Diagnostics Inc., Mannheim, IN) to detect total cholesterol (TC, catalog no. 11875540 216), high density lipoprotein (HDL, catalog no. 04713311 190), low density lipoprotein (LDL, catalog no. 03038807 122), and triglyceride (TG, catalog no. 11876023 216) concentrations. The levels of vitamins A and vitamin E were determined according to the instruction for the high performance liquid chromatography (catalog no. IM 34000, Heimburgstrasse, Munich, Germany). Each sample was tested twice and analyzed at the same time to avoid experimental variations.

Yolk Fatty Acid Analysis

At 38 wks of age, seven eggs were randomly selected per treatment (35 eggs total) and cracked. The collected yolk samples were homogenized and kept at -80 °C until fatty acid profile analysis. Three grams of yolk lipids was extracted with a chloroform:methanol (2:1 vol/vol) solution according to a procedure described by Folch et al. (1957). The samples were homogenized using a shaking incubator for

24 h. Then, a 0.9% NaCl solution was mixed into the samples to separate the lipid fractions. The upper layer was transferred into 15 mL screw-capped tubes and subjected to evaporation using a nitrogen evaporator at 50 °C for 1 h. Subsequently, the samples were thoroughly mixed with a 2-mL solution of 14% boron trifluoride in methanol. Triundecanoate was used as an internal standard and heated at 80 °C for an hour. After cooling, 2 mL of hexane and 5 mL of deionized water were added, and the solution was centrifuged at 3,000 rpm for 10 min (HM-150IV, Hanil Co., Ltd., South Korea). Then, 1 μL of purified methylated fatty acid contained in the hexane layer was transferred into vials for fatty acid quantification with a gas chromatograph (HP7890, Agilent Technologies, Santa Clara, CA, USA). Different fatty acids were separated in a capillary column (SP-2560, 100 m x 0.25 mm x 0.25 μ, Supelco, Bellefonte, PA) at a 100:1 split ratio. The samples were initially held for 4 min at 100 °C. Subsequently, the temperature was ramped to 240 °C at 3 °C/min and held at 240 °C for an additional 3 min. The inlet and detector temperatures were maintained at 225 °C and 285 °C, respectively. Fatty acid (FA) compositions were calculated using Agilent ChemStation software. Relative quantities of fatty acid methyl ester were identified by comparison with retention times of standards (37FAME mix, CLA, Sigma-Aldrich, USA). Values are reported as percentages of total fatty acids. Each sample was analyzed twice to minimize variations.

Statistical Analysis

Data were analyzed in a completely randomized design using the GLM procedure of SAS statistical software package, version 9.1 (SAS Inst., Inc., Cary, NC). Cages were considered as the experimental unit for productive performance and egg quality, whereas an individual hen and egg yolk were the units for nutrient retention, blood metabolites, yolk fatty acid accumulation. Significant differences among dietary treatments were assessed by least-significant difference. The linear

and quadratic effects of LPL levels were also assessed using orthogonal polynomial contrasts for all measurements. The alpha levels used for determining significance were 0.05 and 0.01.

RESULTS AND DISCUSSION

Egg Production and Feed Efficiency

Table 2 summarizes the effects of LPL on the layer performance and feed efficiency of hens from 28 to 38 wks of age. There were no significant differences in BW, egg weight, FI, feed per dozen eggs, cracked and abnormal eggs among treatments during the 10-wk-feeding period. However, hen-day production (P = 0.009) and egg mass (P = 0.005) showed linear responses with increasing levels of LPL in a cumulative period. The hen-day egg production ($P < 0.05$) and egg mass ($P < 0.01$) were significantly improved in hens fed LPL75 compared to CON. Feed conversion ratio also showed linearly decrease with LPL addition (P = 0.039). These results were similar to results reported by Attia et al. (2009) and Mandalawi et al. (2015). The improvement in layer performance and feed efficiency may contribute to various phospholipid functions in enhancing physiological processes of the hen reproductive system (Beemster et al., 2002). This resulted in improved production levels in laying performance during the high-producing period and in a better FCR and greater egg mass. Additionally, the feed per dozen eggs of birds during peak periods tended to decrease as the LPL level increased (P = 0.059), which was comparable with the FCR.

Egg Quality Measurements

As presented in Table 3, the dietary treatments had no significant effects on the Haugh unit, eggshell color score, eggshell breaking strength, eggshell thickness and egg composition ($P > 0.05$). These results agreed with those reported by a

previous study (Mandalawi et al., 2015), in which an increase in lecithin content from 20 to 40 g/kg did not show any effects on eggshell quality. Unlike results of Attia et al. (2009), no linear effects were observed on the Haugh unit. This difference may be due to variability in detecting albumen percentages and albumen heights. However, the feeding of various LPL levels to laying hens significantly increased yolk color score linearly from 6.84 to 8.46 ($P = 0.038$). Feeding LPL75 significantly increased yolk color score compared to CON treatment ($P < 0.05$). This improvement in yolk pigmentation was consistent with the results of Attia et al. (2009) and Mandalawi et al. (2015). The darker yolk color could be influenced by increased crude fat digestibility and vitamin A concentration. These results were also in agreement with those reported by Han et al. (2010), who observed a linear increase in vitamin A deposition from 0.05 to 0.15% in hens fed diets containing lysolecithin. The enriched amount of fat-soluble vitamins has been reported to enhance the uptake of β-carotene and lutein in mice (Baskaran et al., 2003). This means that reproductive hens can utilize carotenoids more efficiently for a greater deposition of lipophilic compounds into the yolk.

Nutrient Retention

The nutrient retention of DM, CP and total ash were not affected by the LPL level in laying hens at 33 wks of age (Table 4). These results were similar to those reported by Attia et al. (2009), who did not find any significant effects on DM and CP retention when supplementing lecithin up to 6% in isocaloric diets for laying hens. On the contrary, the crude fat retention tended to improve as the level of LPL increased ($P = 0.051$). The digestibility of crude fat was significantly greater in LPL100 hens than those fed CON ($P < 0.05$). This improvement was likely due to LPL acting as a source for lipophilic compounds that aid in better digestion and absorption of fat and fatty acids (Jones et al., 1992; Zhang et al., 2011). Recent

research indicated that LPL supplementation levels greater than 0.025% in soybean oil increased crude fat retention to comparable levels as those for increased vitamin A and E concentrations.

Metabolic Profiles of Lipid Metabolism

LPL supplementation showed no significant ($P > 0.05$) effects on blood profiles at 33 wks of age (Table 5). These results were similar to those of a broiler trial reported by Roy et al. (2010). At 38 wks of age, the serum cholesterol tended to decrease ($P = 0.057$) and the serum vitamin E concentration tended to increase ($P = 0.060$) as the LPL levels increased. Hens fed LPL100 showed lower concentrations of TC ($P < 0.05$) and TG ($P < 0.01$) than those fed CON. The reduction of vitamin A concentration was also found in hens fed with CON compared to those fed with LPL75 ($P < 0.05$). The decrease in TC content is associated with the ability to carry cholesterol from peripheral tissues to the liver, which is activated by cholesterol acyl transferase (Rinninger and Pittman, 1987). This mechanism can convert TC and lipids from blood circulation into bile acid for further excretion (Jimenez et al., 1990). Additionally, a linear decrease in triglycerides ($P = 0.020$) and a linear increase in vitamin A ($P = 0.022$) concentrations were observed for increasing LPL levels. The lower triglyceride levels may increase fat digestion rates and overall metabolism. Dietary fats are excreted from the jejunum or may be partially transported from the blood into the ovary to support egg production and yolk pigmentation via increasing fat soluble vitamins.

Yolk Fatty Acid Deposition

The composition of FA in the egg yolk of laying hens fed dietary LPL for 10 weeks is presented in Table 6. The FA percentage of C14:0, C16:0, C16:1, C18:0 and SFA linearly decreased ($P < 0.05$), whereas the FA profiles of C18:3n-3,

C18:3n-6, C20:1, C20:2, and C22:6n-3 linearly increased with the LPL level ($P < 0.01$). However, the dietary treatments did not affect the FA composition of C18:1n-9, C20:3n-6, C20:4n-6, and C24:1n-9. The inclusion of LPL100 significantly increased C18:2n-6 ($P < 0.05$) and decreased SFA ($P < 0.01$) profiles compared to the CON. The supplemental effects of phospholipid for improving desirable FA profiles in poultry products were limited. This study observed the positive effects of LPL on yolk fatty acid profiles. The increase in the levels of C18:2n-6 cis, i.e., the precursor to arachidonic acid (AA; C20:4n-6), has been shown to be beneficial for cardiovascular patients when present in small amounts due to antagonism with n-3 PUFA (Kinsella, 1986). In this study, the level of AA was unaffected by LPL-supplemented groups, indicating that no considerable antagonistic exist. It is expected that LPL may enhance the n-3 PUFA, which further used for increasing the concentrations of eicosapentaenoic and docosahexaenoic acids. These effects are beneficial for fetal development (Innis, 2005) and immune systems (Miles and Calder, 2012). Based on these results, LPL can be supplemented to laying hen to modulate the deposition of PUFA in the yolk.

CONCLUSION

It is indicated that the supplementation of LPL up to 0.10% is sufficient to maximize egg production, egg mass, feed efficiency and yolk pigmentation of laying hens with improvements to fat digestibility. Furthermore, serum cholesterol decreased with increasing levels of LPL. The current study shows that LPL supplementation in laying hen diets results in health benefits for consumers in producing eggs enriched in linolenic acid.

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Table 1. Ingredient and nutrient composition of the experimental diets (% as fed basis)[1]

Ingredient (%)	CON	LPL25	LPL50	LPL75	LPL100
Corn	58.77	58.77	58.77	58.77	58.77
Soybean meal (45%)	22.61	22.69	22.80	22.90	22.99
Rapeseed meal	1.69	1.58	1.45	1.33	1.21
Corn gluten meal (46%)	3.00	3.00	3.00	3.00	3.00
Soybean oil	2.07	2.07	2.07	2.07	2.07
L-Lysine sulfate, (78%)	0.09	0.09	0.09	0.09	0.09
DL-Methionine (98%)	0.17	0.17	0.17	0.17	0.17
Dicalcium phosphate	2.64	2.64	2.64	2.64	2.64
Limestone	8.46	8.46	8.46	8.46	8.46
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Lysophospholipid	0.00	0.025	0.050	0.075	0.100
Calculated values					
ME (kcal/kg)	2,832.00	2,832.00	2,832.00	2,832.00	2,832.00
CP (%)	17.00	17.00	17.00	17.00	17.00
Lysine (%)	0.90	0.90	0.90	0.90	0.90
Met + Cys (%)	0.74	0.74	0.74	0.74	0.74
Calcium (%)	3.79	3.79	3.79	3.79	3.79
Available P (%)	0.43	0.43	0.43	0.43	0.43
Analyzed values (%)					
Crude protein	16.74	16.43	16.59	16.72	16.43
Crude fat	4.37	5.86	5.67	6.02	6.44
Total ash	4.02	4.87	4.76	4.89	4.63

[1] CON – control diet without LPL; LPL25 – LPL at 0.025%; LPL50 – LPL at 0.05%; LPL75 – LPL at 0.075%; and LPL100 – LPL at 0.10%.

[2] Levels supplied per kilogram of diet: vitamin A (from retinyl acetate), 11,000 IU; vitamin D_3, 5,000 IU; vitamin E (from dl-α-tocopheryl acetate), 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; and calcium pantothenic acid, 20 mg.

[3] Levels supplied per kilogram of diet: copper (copper sulfate-pentahydrate), 29 mg; zinc (zinc oxide), 108 mg; manganese (manganese oxide), 115 mg; iron (iron carbonate), 60 mg; and selenium (sodium selenite), 0.4 mg.

Table 2. Effect of dietary lysophospholipid supplementation on productive performance from 28 to 38 wks of age in laying hens[1,2]

Parameters	CON	LPL25	LPL50	LPL75	LPL100	SEM
Initial BW (kg)	1.82	1.81	1.81	1.79	1.82	0.011
Final BW (kg)	1.85	1.85	1.88	1.85	1.88	0.011
Egg production (%)[†]	85.98^{b}	86.21^{b}	87.89^{ab}	88.68^{a}	87.36^{ab}	1.648
Egg weight (g/egg)	62.23	62.41	62.29	62.23	62.14	0.141
Egg mass (g/d/hen)[†]	53.52^{B}	53.80^{AB}	54.69^{AB}	55.16^{A}	54.28^{AB}	0.956
Feed intake (g/d/hen)	113.96	113.96	114.00	114.65	114.01	0.271
FCR (kg feed/kg egg)[*]	2.15	2.13	2.11	2.10	2.12	0.046
Feed per dozen eggs (kg)	1.61	1.60	1.58	1.57	1.58	0.036
Crack eggs (%)[3]	1.58	1.58	1.40	1.45	1.47	0.143
Abnormal eggs (%)[4]	0.30	0.29	0.27	0.29	0.34	0.067

[1] CON – control diet without LPL; LPL25 – LPL at 0.025%; LPL50 – LPL at 0.05%; LPL75 – LPL at 0.075%; and LPL100 – LPL at 0.10%.

[2] Data are the means of six replicates with 14 laying hens per replicate

[3] Cracked eggs include body-checked egg, star crack, pinhole crack, and hairline crack.

[4] Abnormal eggs include small egg (<30 g), misshapen egg, soft-shell egg, yolkless, white-banded egg, slab-sided egg, translucency and wrinkled egg.

[ab] Means with different superscripts in a same row differ significantly ($P < 0.05$).

[AB] Means with different superscripts in a same row differ significantly ($P < 0.01$).

[*] Linear effect ($P < 0.05$).

[†] Quadratic effect ($P < 0.01$).

Table 3. Effect of dietary lysophospholipid supplementation on egg quality from 28 to 28 wks of age laying hens[1,2]

Parameters	CON	LPL25	LPL50	LPL75	LPL100	SEM
Haugh unit	97.24	96.11	98.13	97.38	97.67	0.383
Yolk color*	6.84[b]	7.72[ab]	8.14[ab]	8.57a	8.46[ab]	0.267
Eggshell color	16.28	16.80	16.66	16.56	16.42	0.133
Shell breaking (N)	40.93	39.55	40.68	41.23	41.83	0.418
Shell thickness (mm)	0.386	0.380	0.381	0.382	0.385	0.001
Eggshell (%)	9.36	9.29	9.28	9.42	9.36	0.089
Yolk (%)	23.79	23.93	23.98	24.09	24.04	0.047
Albumen (%)	66.86	66.79	66.74	66.49	66.60	0.101

[1] CON – control diet without LPL; LPL25 – LPL at 0.025%; LPL50 – LPL at 0.05%; LPL75 – LPL at 0.075%; and LPL100 – LPL at 0.10%.

[2] Data are the means of six replicates with 14 laying hens per replicate.

[ab] Means with different superscripts in a same row differ significantly ($P < 0.05$).

[*] Linear effect ($P < 0.05$).

Table 4. Effect of dietary lysophospholipid supplementation on nutrient retention at 33 wks of age laying hens[1,2]

Parameters	CON	LPL25	LPL50	LPL75	LPL100	SEM
DM (%)	72.38	72.25	72.64	72.48	72.13	0.231
CP (%)	63.71	63.90	63.26	65.70	64.26	0.528
Crude fat (%)	80.96[b]	82.06[ab]	83.74[ab]	84.63[ab]	85.20[a]	0.720
Total ash (%)	38.05	38.18	38.33	38.90	38.86	0.607

[1] CON – control diet without LPL; LPL25 – LPL at 0.025%; LPL50 – LPL at 0.05%; LPL75 – LPL at 0.075%; and LPL100 – LPL at 0.10%.

[2] Values are expressed as the means of seven laying hens from each treatment (n = 35).

[ab] Means with different superscripts in a same row differ significantly ($P < 0.05$).

Table 5. Effect of lysophospholipid supplementation on blood metabolites in laying hens[1,2]

Parameters	CON	LPL25	LPL50	LPL75	LPL100	SEM
Vitamin A (μmol/L)						
33 wks of age	2.51	3.09	3.20	3.41	3.82	0.212
38 wks of age*	2.47^{b}	2.87^{ab}	2.96^{ab}	3.40^{a}	3.24^{ab}	0.117
Vitamin E (μmol/L)						
33 wks of age	14.66	18.14	18.74	20.38	20.48	1.775
38 wks of age	6.38	7.86	9.32	10.82	10. 68	0.695
Total cholesterol (mg/dL)						
33 wks of age	179.43	121.60	111.00	106.28	104.21	14.619
38 wks of age	187.04^{a}	121.00^{ab}	113.83^{ab}	108.50^{ab}	97.33^{b}	15.276
High-density lipoprotein (mg/dL)						
33 wks of age	30.54	35.11	34.81	35.28	38.35	2.381
38 wks of age	28.82	30.12	29.62	31.67	32.27	2.656
Low-density lipoprotein (mg/dL)						
33 wks of age	4.40	3.80	3.80	4.60	4.40	0.302
38 wks of age	13.80	12.40	11.60	12.00	10.40	1.171
Triglyceride (mg/dL)						
33 wks of age	1,510.06	1,395.50	1,367.84	1,309.32	1,283.71	33.193
38 wks of age*	1,610.62^{A}	1,462.17^{AB}	1,417.92^{AB}	1,405.96^{AB}	1,350.17^{B}	29.384

[1] CON – control diet without LPL; LPL25 – LPL at 0.025%; LPL50 – LPL at 0.05%; LPL75 – LPL at 0.075%; and LPL100 – LPL at 0.10%.

[2] Values are expressed as the means of six samples analyzed from each treatment (n = 30).

[ab] Means with different superscripts in a same row differ significantly ($P < 0.05$).

[AB] Means with different superscripts in a same row differ significantly ($P < 0.01$).

[*] Linear effect ($P < 0.05$).

Table 6. Effect of dietary lysophospholipid supplementation on yolk fatty acid profiles (% of total fatty acid) on d 70 feeding period[1,2]

Parameters	CON	LPL25	LPL50	LPL75	LPL100	SEM
C14:0†	0.26^{B}	0.26^{B}	0.29^{AB}	0.33^{A}	0.27^{AB}	0.008
C16:0†	26.00^{A}	25.78^{AB}	25.59^{AB}	25.49^{B}	25.63^{AB}	0.055
C16:1†	0.66^{B}	0.74^{B}	1.44^{A}	1.06^{AB}	0.68^{B}	0.083
C18:0*	11.25^{a}	10.80^{ab}	10.92^{ab}	10.13^{b}	10.09^{b}	0.156
C18:1n-9	38.46	38.46	38.92	38.89	38.29	0.153
C18:2n-6	14.43^{b}	15.18^{ab}	14.55^{ab}	15.79^{ab}	16.19^{a}	0.246
C18:3n-6†	0.15^{B}	0.15^{B}	0.18^{AB}	0.21^{A}	0.17^{B}	0.006
C20:1†	0.25^{B}	0.28^{AB}	0.31^{AB}	0.39^{A}	0.34^{AB}	0.016
C18:3n-3†	0.47^{b}	0.47^{b}	0.60^{ab}	0.66^{a}	0.50^{b}	0.025
C20:2†	0.26^{B}	0.27^{B}	0.31^{AB}	0.35^{A}	0.30^{AB}	0.008
C20:3n-6	0.25	0.30	0.29	0.27	0.30	0.010
C20:4n-6	3.61	3.68	3.66	3.32	3.58	0.058
C24:1n-9	1.58	1.59	1.56	1.53	1.68	0.021
C22:6n-3†	2.38^{A}	2.05^{AB}	1.36^{B}	1.60^{AB}	2.01^{AB}	0.110
SFA††	37.52^{A}	36.84^{AB}	36.80^{AB}	35.95^{B}	35.99^{B}	0.168
MUFA	40.94^{b}	41.07^{b}	42.23^{a}	41.86^{ab}	40.97^{b}	0.171
PUFA	21.54^{AB}	22.09^{AB}	20.97^{B}	22.19^{AB}	23.04^{A}	0.226
PUFA:SFA††	0.57^{b}	0.60^{ab}	0.57^{b}	0.62^{ab}	0.64^{a}	0.009

[1] CON – control diet without LPL; LPL25 – LPL at 0.025%; LPL50 – LPL at 0.05%; LPL75 – LPL at 0.075%; and LPL100 – LPL at 0.10%.

[2] Values are expressed as the means of seven eggs analyzed from each treatment (n = 35).

[ab] Means with different superscripts in a same row differ significantly ($P < 0.05$).

[AB] Means with different superscripts in a same row differ significantly ($P < 0.01$).

[*] Linear effect ($P < 0.05$);

[†] Quadratic effect ($P < 0.01$);

[††] Quadratic effect ($P < 0.05$)

Chapter V: Effects of Metabolizable Energy Levels and Lysophospholipid Supplementation on Productive Performance, Egg Quality, Nutrient Retention, and Blood Metabolites of Laying Hens

ABSTRACT: This experiment was conducted to examine the effects of various metabolizable energy (ME) levels with or without lysophospholipid (LPL) supplementation on productive performance, egg quality, nutrient retention, and blood metabolites of laying hens. A total of 360 50-week-old Hy-Line W36 laying hens were subjected to 6 treatments in a 2 × 3 factorial arrangement with 2 levels of LPL (0 and 0.75 g/kg diet) and 3 levels of ME (2,670, 2,750, and 2,830 kcal/kg). Each treatment had 5 replications of 60 laying hens. No interactions were observed for all criteria of laying performance and egg quality. However, the main effect of the LPL significantly decreased egg weight and the cracked egg percentage ($P < 0.05$), as well as increased the yolk color score ($P < 0.0001$). The dietary ME levels did not improve nutrient retention of crude protein, but the LPL supplementation improved ether extract retention ($P < 0.05$) and total ash ($P = 0.083$). Serum concentrations of α-tocopherol, alanine aminotransferase, triglyceride, and high-density lipoprotein were not affected regardless of the interactions or the main factors during both periods. However, the interactions between ME levels and LPL supplementation significantly improved glucose concentration at 58 weeks of age, which reached the highest value in hens fed ME at a 2,750 kcal/kg combination with 0.75 g/kg of LPL supplementation ($P < 0.05$). Furthermore, the LPL addition showed an improvement in retinol ($P = 0.052$) and a reduction in total cholesterol concentrations ($P = 0.075$). Overall, the supplementation of LPL alone can possibly be used in lower-ME diets to eliminate losses of laying performance and increase yolk color through the improvement in nutrient utilization.

Key words: Metabolizable Energy; Lysophospholipid; Laying Hen; Productive Performance; Blood Metabolite

INTRODUCTION

Feeding low-metabolizable energy (ME) has considerable effects on egg production, feed efficiency (Leeson et al., 2001) and production cost in laying hens (dePersio et al., 2015). Previous research has shown that hens increased their feed intake when diets contained a low-energy diet rather than a high-energy diet to ensure energy requirements (Harms et al., 2000). According to Jalal et al. (2007), who demonstrated that decreasing an energy value to 2,810 kcal of ME/kg (in comparison to 2,900 kcal of ME/kg) had no negative effects on egg production, egg mass, and egg weight in laying hens. However, a modern strain of laying hens, such as the Hy-Line W-36, are less capable of adjusting their feed intake when ME value decreased from 2,957 to 2,757 kcal/kg at 33 to 70 weeks of age, resulting in poor egg production (dePersio et al., 2015). It seems that the increased performance of birds can be achieved on the high-energy diet, but such an approach also increases the feed cost (dePersio et al., 2015). Therefore, it is an important issue for egg producers to minimize their feed cost through dietary manipulation.

Lysophospholipid (LPL) is derived from soy lecithin via the function of phospholipase A_2. The addition of LPL has more potential to emulsify fat in an aqueous environment than phospholipids because one fatty acid chain is removed (Joshi et al., 2006). Recent reports found that soy-derived lecithins improved egg weight, feed efficiency (Mandalawi et al., 2015), and fat-soluble vitamin deposition in egg yolks (Han et al., 2010), as well as enhanced hatchability in laying hens (Attia et al., 2009). In broiler chickens, feeding soy lecithin showed positive effects on intestinal absorption (Khonyoung et al., 2015), growth performance, and digestibility of fat and fatty acids (Raju et al., 2011; Zhang et al., 2011)), as well as lower cholesterol fractions (Huang et al., 2008). However, there are few reports has investigated the effects of ME level and LPL supplementation in laying hens' diet during a peak period. Our hypothesis was that LPL might be supplemented as a compensable ME to promote nutrient absorption of lipophilic substances for laying hens. Consequently, the current study was carried out to examine the effects of various levels of

ME with or without supplementation 0.75 g LPL/kg on egg production, egg quality, nutrient digestibility, and blood metabolites in laying hens.

MATERIALS AND METHODS

The study was conducted in a university research farm (Suwon, South Korea). The care and handling of laying hens were performed under guidelines approved by the Animal Ethics Committee of Seoul National University (Gwanak, South Korea).

Experimental Design and Diet

A 2 x 3 factorial arrangement with 2 levels of LPL (0 and 0.75 g/kg) and 3 levels of ME (2,670, 2,750, and 2,830 kcal/kg) was applied in this study. The powdered product of LPL mainly contained 37.5 g/kg of LPL (Easy Bio Inc., Seoul, South Korea). The 6 treatments were as follows: treatments 1 to 3 (T1-T3): basal diets comprising 2,670, 2,750, and 2,830 kcal/kg, respectively, without LPL supplementation; and T4-T6: basal diets comprising 2,670, 2,750, and 2,830 kcal/kg, respectively, with 0.75 g/kg of LPL supplementation. Each treatment consisted of 5 replications of 12 laying hens each.

Laying Hens, Diets and Management

A total of 360 50-week-old Hy-Line W36 laying hens with an average egg rate of 92.31 ± 1.38 were used in the current study. The experimental birds were reared in wire-floored cages whose dimensions were 46.6 x 39 x 50 cm-(height x width x length) with 2 hens per cage. The cages were equipped with a feeder and a nipple drinker to ensure that the hens had free access to experimental feed and fresh water throughout the experimental period. A corn-soybean meal basal diet was given in a mash form for an 8-week feeding. All nutrients met or exceeded the recommendation of commercial brown laying hens (Hy-Line International, 2011), except for the ME concentration. The ME levels were 2,670, 2,750, and 2,830 kcal/kg of feed both in the supplemented and unsupplemented groups

(Table 1). The hens were raised in an evaporative house, with constant ambient temperature of 23 ± 2°C and subjected to artificial light for 16 h (16L:8D). Experimental diets and clean water were offered ad libitum.

Performance Measurements

Eggs were recorded daily at 1500 and classified as either normal or cracked eggs (hairline eggs, body-cracked eggs, and pinhole-cracked eggs), expressed as the percentage of cracked eggs. Egg weight was included for both nondamaged and damaged eggs. Egg production was summarized on a hen-day basis. The egg mass was calculated by multiplying hen-day egg production by egg weight/100. Feed intake (FI) was determined on a replication basis. The feed conversion ratio (FCR) was calculated by dividing the egg mass by the FI value. Egg weight, egg production, egg mass, FI, the FCR, and cracked eggs were monitored in a 14 day-interval and showed as an overall period.

Egg Quality Traits

Thirty eggs from each treatment laid in the last day of the 2-wk interval were randomly chosen based on the average egg weight to determine egg quality traits. The sharp edge of the egg was used to measure eggshell breaking strength using an IMDA digital force reader gauge (MTG-40, Fugihira Industry Co., Ltd., Japan). The eggs were broken and used to determine albumen height in three different positions, presented as the average value using a Multitester device (QCM System, Technical Services and Supplies, Dunnington, York, UK); the Haugh unit and yolk color scores were automatically calculated thereafter. Eggshell thickness was performed by removing the eggshell membrane and measuring it in three different positions on the egg (sharp, equator, and blunt) with a digital micrometer of 0.001 mm (IT-014UT, Mitotuyo Co., Ltd., Kawasaki, Japan). All criteria of egg quality were expressed in an average data.

Nutrient Retention

A determination of nutrient retention was conducted during the last week of experiment using 48 laying hens (eight laying hens per treatment). The hens were selected based upon body weight and egg production, and then placed into individual cage. Chromic oxide (Cr_2O_3) at 5 g/kg diet was used as a marker at the beginning and the end of the metabolic trial. All birds were fed diets twice daily at 0630 and 1830 and had access to feed and fresh water. The experiment was carried out with a 5-day adjustment period followed by a 3-day collection period. Dried feces without the contaminations of feather, scales, and filoplumes were collected every 12 h to eliminate nitrogenous loss. The representative samples of the 3-day collection were pooled for each cage, and moisture content was removed with an air-force oven at 60°C for 72 h; they were then ground in a hammer mill (model Z-I, Retsch, Stuttgart, Germany) for homogenous mixture. The samples of feed and dried excreta were analyzed in triplicate for crude protein (CP) by the Kjeldahl method (method 984.13), ether extract (EE) was analyzed by Soxhlet analysis (method 920.39), and total ash was analyzed using a muffle furnace at 600oC (method 942.05) using the standard procedures of the AOAC (2000). The values of the apparent nutrient retention were calculated using the formulas of (Zhang et al., 2011).

Blood Collection and Lipid Metabolites

At the end of 54 and 58 weeks of age, one layer from each replication was randomly chosen for blood collection. Samples of whole blood (5 mL) were taken through a branchial vein using sterilized needles and syringes and immediately transferred into serum tubes with no anticoagulant. The samples were placed at room temperature for 2 h to separate serum, and thereafter centrifuged at 3,000 x g at 4°C for 5 min. Serum without supernatant was carefully removed into 1.5 mL plastic flacon tubes, which were then stored at -80°C until analysis.

The assays of vitamin A (retinol) and vitamin E (α-tocopherol) were determined using standard protocols of the AOAC (1995). The commercial test kits for the analyses of alanine aminotransferase (ALT), glucose, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL) were purchased from Zhongsheng Biochemical Co., Ltd. (Beijing, China). An automatic biochemical analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA) was used for determination of ALT, glucose, TG, TC, and HDL concentrations using colorimetric enzymatic methods. Each sample was tested in duplicate.

Statistical Analysis

All data were subjected to a two-way ANOVA using the General Linear Model procedure of SAS software (SAS Inst., Inc., Cary, NC, USA) to evaluate the main effects of ME levels and LPL supplementation, as well as their interactions. Cages of 12 laying hens were considered the experimental unit for layer performance and egg quality, whereas individual hen was considered as the experimental unit for nutrient retention and blood metabolites. Significance was defined as significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS

Productive Performance

Results for layer production and feed efficiency from 50 to 58 weeks of age are given in Table 2. There were no interactions between dietary LPL and ME levels on egg weight, egg production, egg mass, FI, the FCR, and cracked eggs. However, a lower egg weight and percentage of cracked eggs, such as hairline eggs, body-cracked eggs, and pinhole-cracked eggs, were found in the LPL-supplemented groups ($P < 0.05$). Additionally, decreased ME levels produced less egg mass ($P = 0.064$), resulting in a poorer FCR ($P = 0.073$).

Egg Quality

As shown in Table 3, there were no significant interactions between ME levels and LPL supplementation on albumen height, Haugh units, eggshell breaking strength, and eggshell thickness throughout the cumulative period. However, a main effect of the LPL supplementation on yolk color score was observed ($P < 0.0001$). It was shown that yolk color increased from 5.63 to 6.77.

Nutrient Retention

Results for the effects of ME levels with or without LPL supplementation on the nutrient retention of the laying hens at 58 weeks of age are presented in Table 4. There were no interactions of dietary ME and LPL supplementation on the nutrient retention of CP, EE, and total ash. However, hens fed diets without LPL supplementation had poorer EE retention than those supplemented with LPL ($P < 0.05$).

Blood Metabolites

The blood metabolites of the laying hens fed diets containing different ME levels and LPL supplementation are given in Table 5 and 6. No interactions or main effects were detected for retinol, α-tocopherol, ALT, TG, TC, and HDL concentrations at both 54 and 58 weeks of age. However, there was a significant interaction between ME level and LPL supplementation on glucose concentration at 58 weeks of age ($P < 0.001$). The highest glucose concentration was observed when hens received the diet containing 2,750 kcal of ME/kg with LPL supplementation (Table 5). Furthermore, an improvement of LPL was detected for retinol and TC concentrations. The concentration of retinol was increased from 3.34 to 3.71 μmol/L at 58 weeks of age ($P = 0.052$; Table 5), whereas the concentration of TC was decreased from 126.78 to 112.00 mg/dL at 54 weeks of age ($P = 0.075$; Table 6) in hens fed diets with LPL supplementation.

DISCUSSION

Interactions between dietary ME and LPL additions to improve the performance of laying hens have not been established. A reduction in egg weight was noted when supplementation with LPL was inconsistent with Attia et al. (2009), who found a significant effect when soy-lecithin was added to laying hens' diet, both isocaloric and extra-energetic diets. The decrease in egg size in this study is possibly associated with increased egg production and egg mass. Interestingly, our study found that the addition of LPL produced a significant reduction in damaged eggs, which may have been influenced by greater copper (Cu) absorption (Roy et al., 2010). Previous research demonstrated that Cu is one of important elements for improving the quality of eggshells (Baumgartner et al., 1978). The function of Cu to improve eggshells has been established as a cofactor of lysyl oxidase, which is normally found in the isthmus throughout the shell gland pouch of a hen's oviduct (Akagawa et al., 1999). The presence of this enzyme can modulate the mechanism to convert lysine to desmosine and isodesmosine, resulting in fewer damaged eggs. Egg weight, egg mass, and the FCR showed to improve by ME inclusion. The observed results were that hens fed a high-ME diet could limit their energy intake, as a result of a decreased FCR, whereas egg mass improved. Previous studies also showed that brown egg-laying hens response to greater egg mass when providing apparent metabolizable energy (AMEn) levels of the diet up to 2,850 kcal/kg (Pérez-Bonilla et al., 2012). These results indicate that added ME has influence on egg mass, and LPL minimize eggshell damages.

The addition of 0.75 g/kg LPL in laying hens diet significantly increased yolk color score ($P < 0.0001$), which was consistent with previous reports (Mandalawi et al., 2015; Attia et al., 2009). The improvement in yolk pigmentation may promote carotenoids' (β-carotene and lutein) absorption in the gastrointestinal tract of the hens to transfer dietary lipids into the yolk, resulting in dark yellow appearance. This positive function was previously confirmed in in vitro study (Baskaran et al., 2003), which

observed that lysophosphatidylcholine plays a vital role in enhancement carotenoids' uptake in the intestinal tract of mice. Recently, Han et al. (2010) also demonstrated that the concentration of fat-soluble vitamins significantly increased when soy-derived lysolecithin was included in laying hens' diets. The improvement in EE digestibility by LPL supplementation seems to be supported by the results of the present study.

Supplementation of various phospholipid sources in low-energy and isocaloric diets has been reported to increase nutrient utilization in both poultry (Jansen et al., 2015) and swine (Zhao et al., 2015). The current study also observed the increased nutrient retention of EE, but also a minimal effect on CP retention, which was in agreement with previous reports (Mandalawi et al., 2015; Attia et al., 2009). The improvement in EE retention from LPL supplementation is enhanced by the lack of one hydrophobic tail, in the aqueous environment (Van Nieuwenhuyzen and Tomás, 2008). In addition, Reynier et al. (1985) reported that lecithin has smaller micelles, which can spontaneously form micelles in the intestinal lumen for a better diffusion rate of lipid and lipophilic substances. In broilers, Zhang et al. (2011) found that birds fed isocaloric diet with 0.5 g per kg of lysophosphatidylcholine significantly increased AMEn digestibility. Based on this finding, it seems that LPL supplementation can be used in low-energy diets as compensable ME, improving fat digestibility for high-producing hens.

At 58 weeks of age, significant interactions between LPL and ME were observed for glucose concentrations, indicating the interaction effect. Blood glucose is commonly used as a good indicator to identify the amount of available glucose for growth and productivity. The increased level of blood glucose by the addition of an emulsifier was previously reported by Roy et al. (2010). This study also found that retinol concentration tended to be increased by the main effect of LPL supplementation, which was in agreement with the observation of Han et al. (2010). This confirms the important function of LPL in improving lipid digestibility via an increased fat-soluble vitamin concentration. Additionally, a tendency for lowering TC was affected by LPL addition. The

anticholesterolemic effect of LPL can be explained by the incorporation of lecithin-acyltransferase (LeBlanc et al., 2003) or a reduction in cholesterol permeability in the epithelial membrane for the inhibition of intestinal cholesterol uptake (Jimenez et al., 1990). This enzyme plays an important role in carrying cholesterol from peripheral tissues to the hepatocyte for further excretion. However, this finding was inconsistent with the results of Han et al. (2010) and Roy et al. (2010). A possible reason for the difference may be the age of the laying hens, their breed, the emulsified source, and the inclusion level. It is indicated that supplementation of 0.75 g/kg LPL in various ME diets decreased TC, and it had an effect of promoting retinol secretion with no toxicity to the liver.

CONCLUSION

The addition of ME and LPL shows interaction effect on glucose availability. The main effect of LPL has greater impacts on yolk color, nutrient digestibility, and blood metabolites with less harmful to the liver.

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Table 1. Formula and nutrient composition of the experimental diets (g/kg as-fed basis)[1]

ME (kcal/kg)/	2,670/0	2,750/0	2,830/0	2,670/	2,750/	2,830/
LPL (g/kg) levels				0.75	0.75	0.75
Ingredient (g/kg)						
Corn	591.7	586.1	580.5	602.4	594.8	578.4
Soybean meal (45%)	220.3	223.0	220.5	211.8	223.1	220.6
Rapeseed meal	38.5	27.1	19.7	38.8	17.4	19.7
Corn gluten meal (46%)	18.2	29.0	31.0	18.2	29.0	30.9
Wheat bran	13.1	9.1	8.4	9.9	8.8	9.2
Soybean oil	0.2	7.4	21.5	0.2	7.9	22.1
L-lysine sulfate (55%)	0.6	0.9	1.0	0.6	0.9	1.0
DL-methionine (98%)	1.6	1.6	1.6	1.6	1.6	1.6
MDCP	22.0	22.0	22.0	22.0	22.0	22.0
Limestone	88.8	88.8	88.8	88.8	88.8	88.8
Vitamin premix[2]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[3]	1.0	1.0	1.0	1.0	1.0	1.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0
Lysophospholipid[4]	0.0	0.0	0.0	0.75	0.75	0.75
Calculated composition[5]						
ME (kcal/kg)	2,670	2,750	2,830	2,670	2,750	2,830
CP (g/kg)	170.0	170.0	170.0	170.0	170.0	170.0
Lysine (g/kg)	9.0	9.0	9.0	9.0	9.0	9.0
Met + Cys (g/kg)	7.3	7.3	7.3	7.3	7.3	7.3
Ca (g/kg)	37.9	37.9	37.9	37.9	37.9	37.9
Available P (g/kg)	4.3	4.3	4.3	4.3	4.3	4.3

[1] Metabolizable energy was calculated from the composition of the diets.

[2] The following quantities of vitamin mixture per kilogram of complete diet were provided: vitamin A (retinyl acetate), 11,000 IU; vitamin D_3, 5,000 IU; vitamin E (dl-α-tocopheryl acetate), 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; and calcium pantothenic acid, 20 mg.

[3] The following quantities of mineral mixture per kilogram of complete diet were provided: Cu (copper sulfate), 29 mg; Zn (zinc sulfate), 108 mg; Mn (manganese sulfate), 115 mg; Fe (ferrous sulfate), 60 mg; and Se (sodium selenite), 0.4 mg.

[4] Lysophospholipid product (Lipidol™) was obtained from Easy Bio Inc., Seoul, South Korea.

[5]Calculated values are on an as-fed basis.

Table 2. Effects of metabolizable energy levels and lysophospholipid supplementation on laying hens' performance[1]

Item	Egg weight (g/egg)	Egg rate (%)	Egg mass (g/d/hen)	FI (g/d/hen)	FCR (kg feed:kg egg)	Cracked eggs (%)
Treatments						
2,670 ME	64.82	84.79	54.96	110.06	2.03	1.12
2,750 ME	66.24	89.26	59.12	109.78	1.87	1.09
2,830 ME	65.71	87.35	57.38	109.22	1.91	1.13
2,670 ME + LPL75	64.82	87.32	56.59	111.02	1.97	0.74
2,750 ME + LPL75	64.75	92.86	60.12	108.10	1.80	0.74
2,830 ME + LPL75	65.28	91.99	60.05	109.42	1.83	0.61
Pooled SEM[2]	0.155	1.109	0.697	0.761	0.031	0.080
ME (kcal/kg)						
2,670	64.82	86.06	55.78	110.54	2.00	0.93
2,750	65.50	91.06	59.62	108.94	1.83	0.92
2,830	65.50	89.67	58.72	109.32	1.87	0.87
LPL (g/kg)						
0	65.59	87.13	57.15	109.69	1.94	1.11
0.75	64.95	90.73	58.92	109.52	1.86	0.70
Source of variance ($P <$ value)						
ME	0.062	0.168	0.064	0.709	0.073	0.941
LPL	0.019	0.108	0.193	0.918	0.252	0.013
ME x LPL	0.069	0.923	0.874	0.796	0.990	0.896

ME = metabolizable energy; LPL = lysophospholipid; FI = feed intake; and FCR = feed conversion ratio.

[1] The treatments contained 2 levels of LPL (no addition LPL or 0.75 g/kg) and 3 levels of ME (2,670, 2,750, and 2,830 kcal/kg); [2] Pooled SEM = standard error of the mean.

Table 3. Effects of metabolizable energy levels and lysophospholipid supplementation on laying hens' egg quality[1,2]

Item	Albumen height (mm)	Haugh unit	Yolk color	Shell strength (N)	Shell thickness (mm)
Treatments					
2,670 ME	8.75	91.87	5.50	32.87	0.386
2,750 ME	8.66	91.48	5.65	35.37	0.392
2,830 ME	9.06	92.61	5.75	38.33	0.390
2,670 ME + LPL75	8.74	91.43	6.30	37.40	0.396
2,750 ME + LPL75	8.54	90.70	7.00	39.07	0.390
2,830 ME + LPL75	8.58	90.55	7.00	35.05	0.391
Pooled SEM[3]	0.099	0.522	0.142	0.814	0.002
ME (kcal/kg)	8.75	91.87	5.50	32.87	0.386
2,670	8.74	91.65	5.90	35.14	0.391
2,750	8.60	91.09	6.33	37.22	0.391
2,830	8.82	91.58	6.38	36.69	0.391
LPL (g/kg)					
0	8.82	91.98	5.63	35.52	0.389
0.75	8.62	90.89	6.77	37.17	0.393
Source of variance ($P <$ value)					
ME	0.679	0.905	0.086	0.537	0.994
LPL	0.333	0.336	<0.0001	0.302	0.492
ME x LPL	0.627	0.823	0.437	0.103	0.614

[1] The treatments contained 2 levels of LPL (no addition LPL or 0.75 g/kg) and 3 levels ME (2,670, 2,750, and 2,830 kcal/kg).

[2] Values are expressed as the means of 30 eggs collected from each treatment.

[3] Pooled SEM = standard error of the mean.

Table 4. Effects of metabolizable energy levels and lysophospholipid supplementation on nutrient retention (%) of laying hens[1,2]

Item	Crude protein	Ether extract	Total ash
Treatments			
2,670 ME	57.66	67.80	61.37
2,750 ME	59.91	68.93	62.44
2,830 ME	58.42	70.77	62.95
2,670 ME + LPL75	57.67	74.60	65.05
2,750 ME + LPL75	60.16	79.93	66.78
2,830 ME + LPL75	59.82	77.48	65.99
Pooled SEM[3]	1.138	1.862	1.009
ME (kcal/kg)			
2,670	57.66	71.20	63.21
2,750	60.04	74.43	64.61
2,830	59.12	74.12	64.47
LPL (g/kg)			
0	58.66	69.16	62.25
0.75	59.22	77.33	65.94
Source of variance ($P <$ value)			
ME	0.726	0.733	0.830
LPL	0.820	0.034	0.083
ME x LPL	0.969	0.863	0.967

[1] The treatments contained 2 levels of LPL (no addition LPL or 0.75 g/kg) and 3 levels ME (2,670, 2,750, and 2,830 kcal/kg).

[2] Values are expressed as the means of eight laying hens from each treatment.

[3] Pooled SEM = standard error of the mean.

Table 5. Effects of metabolizable energy and lysophospholipid supplementation on blood metabolites[1,2]

	Retinol (μmol/L)		α-tocopherol (μmol/L)		ALT (U/L)		Glucose (mg/dL)	
Item	54 wks	58 wks	54 wks	58 wks	54 wks	58 wks	54 wks	58 wks
Treatments								
2,670 ME	2.41	3.28	8.60	9.58	5.00	11.32	161.59	177.76
2,750 ME	2.71	3.46	9.65	12.25	4.67	9.45	174.61	180.60
2,830 ME	2.64	3.28	9.59	11.32	4.83	7.69	188.33	197.40
2,670 ME + LPL75	2.64	3.46	9.49	10.57	4.50	9.91	173.17	193.20
2,750 ME + LPL75	3.17	4.01	12.06	12.45	4.58	5.83	197.58	217.01
2,830 ME + LPL75	2.99	3.66	11.76	12.73	4.72	6.89	193.62	183.60
Pooled SEM[3]	0.111	0.095	0.667	0.718	0.204	1.239	8.122	2.938
ME (kcal/kg)								
2,670	2.52	3.37	9.04	10.08	4.75	10.62	167.38	185.48
2,750	2.94	3.73	10.85	12.35	4.62	7.64	186.09	198.80
2,830	2.82	3.47	10.68	12.03	4.78	7.29	190.98	190.50
LPL (g/kg)								
0	2.59	3.34	9.28	11.05	4.83	9.49	174.84	185.26
0.75	2.94	3.71	11.10	11.92	4.60	7.55	188.12	197.94
Source of variance ($P <$ value)								
ME	0.306	0.260	0.499	0.416	0.954	0.522	0.494	0.040
LPL	0.126	0.052	0.192	0.567	0.594	0.459	0.439	0.004
ME x LPL	0.911	0.715	0.888	0.945	0.913	0.897	0.911	<0.001

ME = metabolizable energy; LPL = lysophospholipid; and ALT = alanine aminotransferase.

[1] The treatments contained 2 levels of LPL (no addition LPL or 0.75 g/kg) and 3 levels ME (2,670, 2,750, and 2,830 kcal/kg).

[2] Values are expressed as the means of five laying hens analyzed from each treatment.

[3] Pooled SEM = standard error of the mean.

Table 6. Effects of metabolizable energy levels and lysophospholipid supplementation on serum cholesterol fractions[1,2]

	TG (mg/dL)		TC (mg/dL)		HDL (mg/dL)	
Item	54 wks	58 wks	54 wks	58 wks	54 wks	58 wks
Treatments						
2,670 ME	861.06	1,077.40	121.33	144.87	13.56	12.83
2,750 ME	902.83	1,177.43	126.33	143.15	14.58	11.24
2,830 ME	981.10	1,296.89	132.67	147.83	14.65	13.26
2,670 ME + LPL75	764.75	911.50	112.83	126.23	14.48	13.57
2,750 ME + LPL75	841.50	1,167.27	102.50	130.80	15.91	14.41
2,830 ME + LPL75	881.74	1,192.28	120.67	135.71	15.69	14.83
Pooled SEM[3]	28.266	52.84	4.046	4.701	0.787	0.772
ME (kcal/kg)						
2,670	812.91	994.4	117.08	135.55	14.02	13.20
2,750	872.17	1,172.4	114.42	136.97	15.25	12.83
2,830	931.42	1,244.6	126.67	141.77	15.17	14.05
LPL (g/kg)						
0	915.00	1,183.9	126.78	145.28	14.26	12.45
0.75	829.33	1,090.4	112.00	130.91	15.36	14.27
Source of variance ($P <$ value)						
ME	0.244	0.155	0.431	0.862	0.801	0.820
LPL	0.138	0.382	0.075	0.151	0.517	0.266
ME x LPL	0.954	0.833	0.717	0.953	0.995	0.823

ME = metabolizable energy; TG = triglyceride; TC = total cholesterol; and HDL = high density lipoproteins.

[1] The treatments contained 2 levels of LPL (no addition LPL or 0.75 g/kg) and 3 levels ME (2,670, 2,750, and 2,830 kcal/kg).

[2] Values are expressed as the means of five laying hens analyzed from each treatment.

[3] Pooled SEM = standard error of the mean.

Chapter VI. Overall Conclusion

Feed cost is a major contribution in livestock production worldwide. The studies were aimed to increase nutrient utilization of broiler chickens and laying hens by the use of biosurfactant, lysophospholipid (LPL, Lipidol^{TM}). The hypotheses were that supplementation LPL will be used as compensable sources of energy, protein including amino acid either in lower or adequate nutrient diets. This approach would be validated for poultry producers' to minimize feed cost with no retardation on growth performance and laying productivity. Consequently, three experiments were performed. The first experiment investigated the effects of supplementation of LPL in lower nutrient diets on growth performance, blood metabolites, immunity, and gastrointestinal morphology in broiler chickens. The results showed that feeding low nutrient diets with no addition of LPL had detrimental effects on growth performance, decreased nutrient retention, susceptibility of inflammatory responses, and less accumulation of meat muscle. However, the inclusion of 0.05% LPL had greater impacts on broiler performance. In addition, the LPL addition is related to activating metabolic profiles of carbohydrate, protein, and lipid via increased glucose concentration, decreased free fatty acid and serum uric acid concentrations. The greater available of glucose and lower uric acid concentrations cause further metabolites and ultimately contributes to the availability of energy and amino acids in low nutrient diets. The LPL also acts as an immunostimulant by lowed concentrations of tumor necrosis factor alpha and interleukin-1 which consistently results of shorter crypt depth. The positive effects of LPL on performance, nutrient digestibility, blood metabolites, intestinal morphology, and carcass characteristics were obviously demonstrated that 0.05% of LPL was the proper level for broilers fed reduced energy, crude protein including some essential amino acids' diets.

The second experiment examined the effects of dietary LPL supplementation on layer performance, egg quality, nutrient digestibility, blood related lipid metabolism, yolk

fatty acid deposition of brown egg-laying hens from 28 to 38 wk of age. Results showed linear improvements in hen-day egg production, egg mass, and reductions in feed conversion ratio (FCR) and feed per dozen eggs significantly detected in groups fed LPL. Dark-yolk pigmentation and fat digestibility increased numerically by the inclusion of LPL supplementation. Serum triglyceride and retinol concentrations were significantly reduced at 38 weeks of age. Yolk polyunsaturated fatty acids (PUFA), linolenic acid, monounsaturated fatty acids linearly increased in groups fed LPL.

The third experiment examined the effects of metabolizable energy (ME) level and LPL supplementation on productive performance, egg quality, nutrient retention, and blood metabolites of laying hens. The results showed inclusion level of LPL at 0.075% decreased egg weight, cracked egg percentage as well as increased yolk color score. The LPL supplementation also had positive effects on crude fat digestibility and a tendency for ash digestibility. Concentrations of serum retinol and total cholesterol were changed in response to the LPL supplementation. However, the ME had little effects on laying performance, except for increased egg weight and lowered FCR. Interestingly, the interactions between ME level and LPL supplementation were observed on glucose concentration at 58 wk of age. It reached the highest value in hens consumed ME at 2,750 kcal/kg combination with 0.075% of LPL supplementation. It is indicated that the supplementation of LPL alone can possibly be used in lower-ME diets to eliminate losses of laying performance and increase yolk color through the improvement in nutrient utilization.

Overall, the addition LPL in standard and lower nutrient diets could be applied in poultry production. It resulted in promising growth performance, nutrient retention, anti-inflammation, and lowering-cholesterol fractions and yolk saturated fatty acids without toxicity to the liver. For these reasons, the LPL would be an alternative biomaterial to improve growth performance of broiler chickens and egg production of laying hens.

Chapter VII. Summary in Korean

본 연구의 목적은 lysophospholipid (LPL)을 이용하여 육계와 산란계에서 영양소의 이용률을 높이는데 있다. 이 연구는 양계 농가에게 성장 정체와 산란율 저하를 유발하지 않으며 사료비를 최소화할 수 있는 방법을 제시하고자 다음 세 가지 실험이 수행되었다.

Experiment I. Effects of Lysophospholipid Supplementation to Lower Nutrient Diets on Growth Performance, Intestinal Morphology, and Blood Metabolites in Broiler Chickens

본 연구는 저에너지 및 저단백질 사료 내 lysophospholipid (LPL)의 첨가가 육계의 성장 성적, 장벽 발달, 혈액 성상, 염증 반응 및 도체 특성에 미치는 영향을 알아보기 위해 수행되었다. 총 300 마리의 1일령 수평아리 (Ross 308)를 대상으로 실험을 개시하였으며, 임의블록배치법에 의해 5처리 6반복 반복당 10 수씩 구배치하였다. 각 처리구는 다음과 같다. Positive control (PC) – 영양소 요구량에 맞춘 LPL이 없는 처리구; negative control (NC) – 영양소 요구량 보다150 kcal/kg의 대사에너지가 낮고 조단백, 아미노산 (Lys, Met, Thr, and Trp) 수준을 5%씩 낮춘 처리구; LPL05 – NC처리구 기초사료에 0.05% LPL첨가한 처리구; LPL10 – NC 처리구 기초사료에 0.10% LPL 첨가한 처리구; LPL15 – NC처리구 기초사료에 0.15% LPL 첨가한 처리구. NC처리구 사료를 급여한 육계의 성장성적과 가슴근육의 무게가 PC 처리구에 비해 낮게 나타났다. 또한 TNF-α (P = 0.011), interleukin-1 (P = 0.036)의 분비와 공장과 십이지장의 융와가 증가하는 NC처리구가 염증반응에 더 민감하게 반응하는 것으로 나타났다. 그러나 사료 내 LPL의 첨가수준이 증가할수록 육계의 성장성적, 사료효율, 지방 및 단백질 축적이 향상되었다. 저영양소 사료에 LPL의 첨가시 유의적은 낮은 uric acid가 나타났다 (P = 0.001). 더욱이 NC 기초사료에 LPL

을 첨가한 처리구들의 십이지장의 융와(P = 0.074)와 TNF-α (P = 0.082)가 감소하여, 염증반응을 감소하는 효과를 가지는 것으로 나타났다. 이러한 결과는 도체특성에서 췌장과, 가슴근육, 다리근육의 상대적 무게에도 효과를 미치는 것으로 나타났다. 반대로, LPL의 급여는 면역기관과 근위, 복강지방의 상대적 무게에는 유의적인 영향을 주었다. 전체적으로 저 에너지 및 단백질사료에 LPL의첨가는 육계의 성장성적과 영양소 소화율, 장건강, 항염증반응 및 근육생산을 향상시킨다.

Experiment II. Effects of Dietary Lysophospholipid Supplementation on Egg Production, Lipid Metabolism, Yolk Fatty Acid Deposition in Brown Egg-Laying Hens

본 실험은 사료 내 lysophospholipid (LPL)의 첨가가 28 – 38 주령의 산란계의 산란율, 난 품질, 지방 대사 및 난황 내 지방산 조성에 미치는 영향을 조사하기 위해 수행되었다. 총 420수의 Hy-Line W36 산란계를 5처리 6 반복, 반복당 14마리로 임의완전블록배치법에 의해 구배치하였다. 산란계들은 처리구에 따라 각각 0 (CON), 0.025 (LPL25), 0.05 (LPL50), 0.075 (LPL75)과 0.10% (LPL100)의 LPL이 첨가된 실험사료를 급여하였다. 산란성적과 계란 품질에 있어 처리구간의 유의적인 차이는 발견되지 않았다. 그러나, LPL 수준이 증가함에 따라 일계산란율(P=0.009)과 난무게(P = 0.005)가 증가하는 결과가 나타났다. LPL75처리구는 대조구에 비해 일계산란율(P < 0.05), 난무게(P < 0.01), 난황색(P<0.05)가 더 높게 나타났다. 또한 LPL 첨가수준이 증가함에 따라 난황층이 더 진해지는 결과가 나타났다 (P = 0.038). 영양소소화율을 측정한 결과, 건물, 조단백, 조회분의 소화율에는 처리구간의 유의적 차이는 나타나지 않았다. 그러나 LPL의 첨가수준이 증가함에 따라 지방축적이 증가하는 결과가 나타났다 (P = 0.051). 33주령의 산란계의 콜레스테롤, 비타민A, 비타민E의 농도는 처리구의 영향을 받지 않았다. LPL100 처리구는 대조구에 비해

낮은 콜레스테롤 농도를 가지고 있었으며, 높은 C18:2n-6와 PUFA:SFA 비율을 가지는 것으로 나타났다($P < 0.05$). LPL의 첨가는 포화지방산의 축적비율을 감소시켰다 (linear effects; $P = 0.03$). 결론적으로 산란계 사료내 LPL의 첨가는 콜레스테롤농도를 감소시키고 산란성적을 개선하는 것으로 사료된다.

Experiment III. Effects of Metabolizable Energy Levels and Lysophospholipid Supplementation on Productive Performance, Egg Quality, Nutrient Retention, and Blood Metabolites of Laying Hens

본 연구는 여러 대사에너지 (ME)수준에 따른 LPL의 첨가 효과가 산란계의 생산성, 계란 품질, 영양소 소화율 그리고 혈액 성상에 미치는 영향을 조사하기 위해 수행하였다. 50 주령의 Hy-Line W36 산란계 360 수를 공시하여, 2 × 3 요인설계로 LPL 첨가유무에 따른 요인(0, 0.75 g/kg diet)과 에너지수준(2,670, 2,750, 2,830 kcal/kg) 요인으로 처리구를 설정하였다. 실험동물들은 각각의 처리구에 5반복씩, 60 수의 산란계가 구배치되었다. 산란성적과 난품질에서 에너지와 LPL요인에 따른 유의적인 효과는 나타나지 않았다. 그러나 LPL의 첨가는 난무게를 감소시키고 파란율을 감소키며($P < 0.05$), 난황색도 증가하였다 ($P < 0.0001$). 사료내 에너지수준은 조단백의 축절을 향상시키지는 못하였지만 LPL의 첨가는 조지방 ($P < 0.05$) 및 조회분 ($P = 0.083$)의 소화율을 향상시켰다. 혈중 α-tocopherol, alanine aminotransferase, triglyceride, and high-density lipoprotein은 요인에 따른 유의적인 차이가 나타나지 않았다. 하지만 에너지수준과 LPL첨가에 따른 상호작용이 58주째의 혈중 glucose 농도를 증가시켰으며, ME 2,750 kcal/kg에 LPL 0.075g/kg을 첨가하였을 때 가장 높은 값을 가졌다($P < 0.05$). 또한 LPL의 첨가는 retinol을 향상시키고($P = 0.052$) cholesterol 농도를 감소시켰다 ($P = 0.075$). 결론적으로 LPL의 첨가는 저에너지수준은 산란성적은 저하를 방지하고, 영양소소화율을 개선하여 난황색을 향상시키는 것으로 사료된다.