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A Thesis

For the Degree of Master of Science

**Efficiency of Sodium Stearoyl-2-lactylate as  
an Exogenous Emulsifier Supplementation  
on Growth Performance, Nutrient  
Digestibility and Blood Component in  
Weaning Pigs**

사료내 유화제로서 SSL의 첨가가 이유자돈의  
성장성적, 영양소 소화율 및 혈액 성상에  
미치는 영향

August, 2012

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# Efficiency of Sodium Stearoyl-2-latilate as an Exogenous Emulsifier Supplementation on Growth Performance, Nutrient Digestibility and Blood Component in Weaning pigs

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미치는 영향

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이 논문을 농학석사 학위논문으로 제출함




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# Summary

The ingredients market for animal feed industry has steadily increased in last decades. And environment concern about animal manure has led to an approach to find out the methods to increase digestibility of feedstuff. Especially, fat has been regarded as the most expensive ingredient but excellent energy source in swine diet. However, dietary fat enrichment is difficult in the early weaned pig because of digestive limitations. It is known that digestive enzyme's secretion in GI tract in weaning pig is not enough to digest grain source ingredients. An experiment was conducted to evaluate the efficiency of sodium stearyl-2-lactylate as an emulsifier on growth performance and nutrient digestibility and blood component in weaning pigs. A total of 128 crossbred ([Yorkshire × Landrace] × Duroc) pigs, averaging  $7.07 \pm 1.52$  kg initial body weight were randomly assigned based on sex and body weight in a randomized complete block(RCB) design in 8 replicates with 4 pigs per pen. The 2 x 2 factorial design was used in this experiment and the first factor was supplementation levels of fat (no fat added or 2% fat added diet), and the second factor was two levels of emulsifier (diets containing 0 or 0.1% of SSL). Experiment was conducted with corn-soy bean meal based diet and two phase feeding programs were used. The phase I diet was provided for the first 2 weeks and the phase II diet was given for last 3 weeks. During the whole experimented period(0~5week), no significant improvement of body weight and G:F ratio was observed by dietary fat or emulsifier treatment. However, there were tendency of fat effect on ADFI (P=0.09) and emulsifier effect on G:F ratio (P=0.08). It is noted that added fat in weaning pigs diet affected on lowered ADFI but emulsifier supplementation in the diet increased feed efficiency. In nutrient digestibility trial, crude fat digestibility tended to improve approximately 5% more than that of

non-emulsified treatment( $P=0.1$ ). Exogenous emulsifier(SSL) may improving the digestibility of fat in diet. As a result of digestibility of fat, HDL:LDL ratio was changed in blood analysis( $P<0.05$ ). Consequently, this experiment demonstrated that exogenous emulsifier(SSL) is able to increase fat digestibility in weaning pigs particularly 2 weeks postweaning.

**Key words : Weaning pig, Emulsifier, SSL, Sodium stearyl -2- lactilate, Growth performance, Nutrient digestibility**

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## List of Abbreviation

AOAC	:	Association of official analytical chemists
ADG	:	Average daily gain
ADFI	:	Average daily feed intake
BW	:	Body weight
CMC	:	Critical micelle concentration
CP	:	Crude protein
FCR	:	Feed conversion ratio
GI	:	Gastrointestinal
HLB	:	Hydrophile-lipophile balance
LCFA	:	Long chain fatty acids
MCFA	:	Medium chain fatty acids
ME	:	Metabolizable energy
NRC	:	National research council
RCB	:	Randomized complete block
SAS	:	Statistical analysis system
SBM	:	Soybean meal
SSL	:	Sodium stearyl-2-latilate

# I. Introduction

Currently corn, wheat and soybean meal are widely used as primary ingredients for energy and protein sources in swine feedstuff. However, the price of international major feed ingredients had been increased since 2006 because of increasing meat consumption in developing countries such as china as well as for biofuel production and financial issue around the world. In a time with a very high feedstuff price, many swine producers and nutritionists have been searched for strategies to reduce the feed cost with minimally sacrificing animal's productivity.

Several methods to reduce feed cost and increase animal performance had been searched by nutritionist and one of the way was improving digestibility. In that method, feed producer studied particle size and processing methods such as pelleting and extruding of grain feedstuff. Various kinds of feed ingredient were used in swine diet, but the digestibility of every ingredients has huge difference relating to their contents of nutrients and feed additive which can increase the feed digestibility. Especially fat source was very good ingredients just for energy enhancement in animal feeds. So, many studies were focused on how to increase fat digestibility in feedstuff.

The dry matter of sow's milk contains about 35% fat (Frobish et al., 1969), it represented that young pigs could utilize fat very well. At weaning, piglet has been suffering from serious change, such as enzyme secretion and its activity. According to Jensen et al. (1997) the development of lipase, colipase, and carboxyl ester hydrolase activity decreased post-weaning, whereas gastric lipase activity increased before weaning and remained constant regardless of weaning.

Therefore, feed additives such as an emulsifier has investigated as

surfactant of fat source in the diet. A typical example of emulsifier, lecithin is widely used both in food and feed industries. A lot of researches found the effect of lecithin on performance of young pigs was inconsistent and limited (Frobish et al., 1969; Kanyo et al., 1985; Van Wormer and Pollman, 1985; Jones et al., 1990; Jones et al., 1992). While some authors indicated a potential benefit (Jones et al., 1992), others reported no positive responses on digestibility (Overland et al., 1993, 1995). Lysolecithin was also introduced as an emulsifier in weaning pigs and broilers (Rodas, 1995; Danek et al., 2005; Zhang et al., 2011).

Sodium stearoyl-2-lactylate(SSL) is O/W (oil in water) type emulsifying agent which is readily dissolved in water and allows fat to be readily involved in the aqueous phase in the body. However, only a few studies were conducted and researches are concentrated primarily on lecithin (W/O; water in oil) which resulted inconsistently on weaning pig's growth performance (Frobish et al., 1969; Kanyo et al., 1985; Van Wormer and Pollman, 1985; Jones et al., 1990).

The aim of the present research was to test the effects of SSL as an emulsifier agents on nutrient digestibility, blood analysis and growth performance in weaning pigs.

## II. Review of Literature

### 1. Introduction

#### 1.1 Global market and feed ingredients

World grain prices increased dramatically in 2007 and the 1st and 2nd quarter of 2008 creating a global financial crisis and causing political and economical instability and social unrest both in poor and developed nations. Even though the world grain market is generally unstable, bio-ethanol policies of grain export countries and an inflow of speculative funds have made the food crisis worse (Yoon, 2008). After that feed crisis in 2007 and 2008, market prices of major feed ingredients kept high in recent years. If a major feed grain price increased 10%, it will affect more than 10% on by-products which are common used in feed industry. If corn, wheat, and soy prices together increased 100% at the same time, this will raise the consumer price index approximately 0.7%, affect household spending, and burden the economy (Kim, 2011).

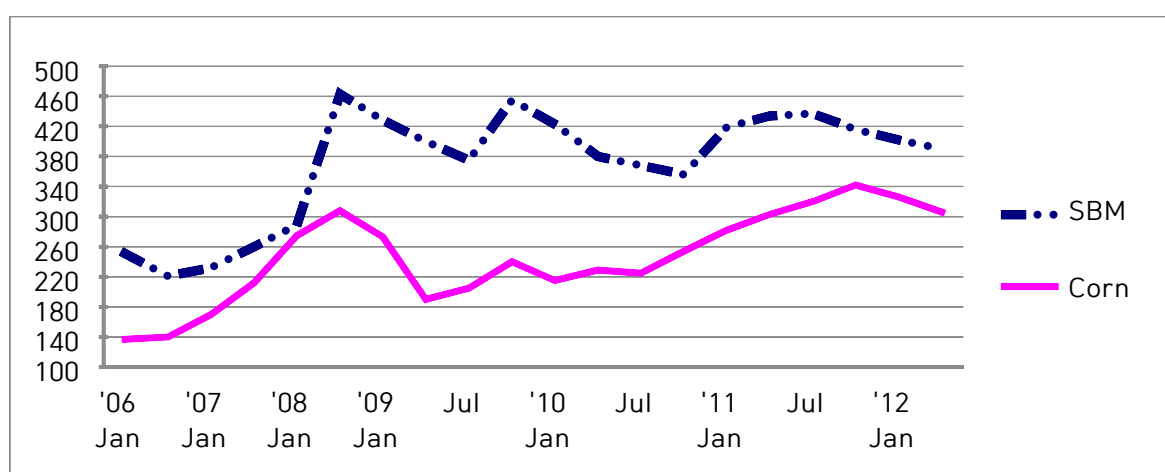


Figure 1. Corn and SBM import price in Korea (Korea Feed Association, 2012)

In recent years, a third popular explanation was rapid economic growth in certain emerging economies, notably China and India, increasing demand for food, especially for livestock products, which generated increased cereal and oilseed demand for feed (FAO, 2009). Consequently, the world's grain demand had to increase by 40-50 percent, driven strongly by rapidly growing economic animal feed use and meat production. Recorded crude oil prices and environmental concerns strengthened interest in alternative energy sources and policy measures in the United States of America, and the European Union (EU) encouraged the expansion of biofuel production (FAO, 2009). Thereby, world grain commodities is thought to maintain higher prices because of those reasons above mentioned.

## **1.2 Environmental concern about livestock industry in Korea**

Nutrition has important effects on the environment as a consequence of the process of digestion (methane and phosphorus) or a combination of digestion and metabolism (nitrogen) (Mc Donald et al., 2011). Livestock manure was one of important resources of fertilizers in traditional agriculture. Unless it disposed appropriately, it became a factor of environment pollution (Ha, 2010). As the main part of nitrogen in manure, ammonium was readily oxidized to nitrate that was poorly absorbed by soil colloids, thus making it easy to move into surface waters (Luo et al., 2002).

Total nitrogen and phosphorus from pig industry increased continuously. In Korea, almost 40,640 metric ton of nitrogen and 7,195 metric ton of phosphorus were excreted soil and ocean from pig industry in 2009 (Ha, 2010). From 2012, Korean government prohibited the ocean dumping of livestock manure. After that, nutrient digestibility of feedstuff would be more important issue in livestock industry of Korea for protecting domestic environment.

Table 1. Population of pig and emission of nitrogen and phosphorus in Korea

(unit : ,000 pig, MT/year)

Year	Population End of year	Nitrogen			Phosphorus		
		Feces	Urine	Total	Feces	Urine	Total
1965	1,382	2,979.8	2,879.8	5,859.6	944.4	93.0	1,037.4
1970	1,126	2,427.8	2,346.3	4,774.1	769.5	75.8	845.3
1975	1,247	2,688.7	2,598.5	5,287.2	852.2	83.9	936.1
1980	1,784	3,846.6	3,717.4	7,564.0	1,219.1	120.1	1,339.2
1985	2,853	6,151.5	5,945.0	12,096.5	1,949.6	192.1	2,141.7
1990	4,528	9,763.1	9,435.3	19,198.4	3,094.3	304.8	3,399.1
1995	6,461	13,931.0	13,463.2	27,394.2	4,415.2	435.0	4,850.2
2000	8,214	17,005.7	16,434.6	33,440.3	5,389.7	531.0	5,920.7
2005	8,961	19,322.5	18,673.7	37,996.2	6,124.0	603.3	6,727.3
2009	9,584	20,666.6	19,972.7	40,639.3	6,550.0	645.3	7,195.3

(Ha, 2010)

## 2. Growth of the weaned pig

### 2.1 Changes of digestive physiology in weaned pigs

The GI (gastrointestinal) tract of mammals includes the mouth and associated structures and glands, the esophagus, the stomach, the small intestine, and the large intestine (including a cecum in some species) (Pond et al., 2005). Investigations of the interactions between pre-and post-weaning nutrition, gut physiology and immunology and their relevance to bodily functions and health are fundamental to resolve the problems of dietary change and post-weaning performance (Kelly and King, 2001). Post weaning feed intake inadequacies together with stress and disease challenge ensure a rapid loss of body lipid in support of maintenance and protein synthesis (Whittemore and Green, 2001). Piglets suffering post-weaning stress showed a



reduced feed intake and a shift in the partitioning of dietary nutrients away from skeletal muscle development toward a metabolic response to support the immune system, resulted in accelerating lipolysis and muscle protein degradation. In these case animals may even suffer a net loss in weight rather than increase in weight and growth. Also this weaning stress could be reason for ADG different between actual and potential.

According to previous investigations, sucking pigs grow at approximately 220g /day between birth and weaning, but this growth rate was far below the biological potential of the artificially reared pig, which can grow in excess of 400g/ day (Hodge, 1974; Dunshea et al., 1999). The liveweight at weaning was greater in pigs that were larger and older but was not different between boars and gilts, liveweight increased with time after weaning with effects of sex, age, and weight being maintained (Pluske et al., 2003).

According to the Pluske et al., (2003) the weight of the organs (liver, heart, kidneys, pancreas) were greater in pigs that were larger and older at weaning, but were not different between boars and gilts. The growth of organs related with digestion cannot be affected by weaning. However, Hampson (1986) suggested that weaned pigs have highly significant increases in crypt depth and increased complexity of villus morphology with a dramatic reduction in villus height compared to unweaned pigs. And several investigations (Kenworthy, 1976; Pluske et al., 1996) also reported a reduction in villus height and an increase in crypt depth after weaning. The results of the experiment by Kelly et al., (1991), pigs were weaned at 14 days and fed a weaning diet by gastric incubation(force feeding), would suggest that the deleterious changes in small intestinal morphology and carbohydrase enzyme activities which occurred immediately after weaning are in part due to low post-weaning feed intakes. These morphological responses

to weaning have a great effect on mucosal functions in the small intestine. Several reports clearly observed that villus atrophy and crypt hyperplasia was usually associated with a decline in the activities of the brush border enzymes (Hampson and Kiddler, 1986; Miller et al., 1986).

## **2.2 The change of digestive enzymes**

The small intestine of the early-weaned pig undergoes major changes in villous architecture and reductions in specific enzyme activity during the immediate post-weaning period (Armstrong & Clawson, 1980). According to Corring et al., (1978), activities of lipase, amylase, trypsin and chymotrypsin undergo a two-step development; increasing steadily from birth to the third week and more rapidly from the fourth to the eighth week of age. It was also stated that there was a high correlation between pancreas : bodyweight ratio and growth, which increased in the first week and was more rapid from the fourth week of age. The gastrointestinal system has to adapt to the considerable changes in the physicochemical properties of their feed as well as to a change in the pattern of intake in order to satisfactorily digest and absorb the nutrients in the diet and maintain an acceptable growth rate (Jensen et al., 1997).

The activity of lactase was high at birth and reaches a maximum in the first week of life and then slowly declined over the third or fourth week and maltase activity increases from the fourth week, while sucrase reaches a constant level between weeks 4 and 8. The activity of  $\alpha$ -amylase was present at birth but remained low until about 4 weeks of age (Mc Donald et al., 2011). Cera et al. (1990b) reported that pancreas weights from suckling pigs increased linearly ( $P < 0.01$ ) from 2 to 35 day of age and weaned at 21 day of age had pancreas weights that declined at 3 day post-weaning and were lower than for suckling pigs at 7 day but increased linearly thereafter. At the

weaning time lipids digestibility in weaning pig have serious change from enzyme secretion (Cera et al., 1990b; Jensen et al., 1997). During the suckling period the activity of carboxyl ester hydrolase was high in order to take part in the digestion of milkfat (Jensen et al., 1997).

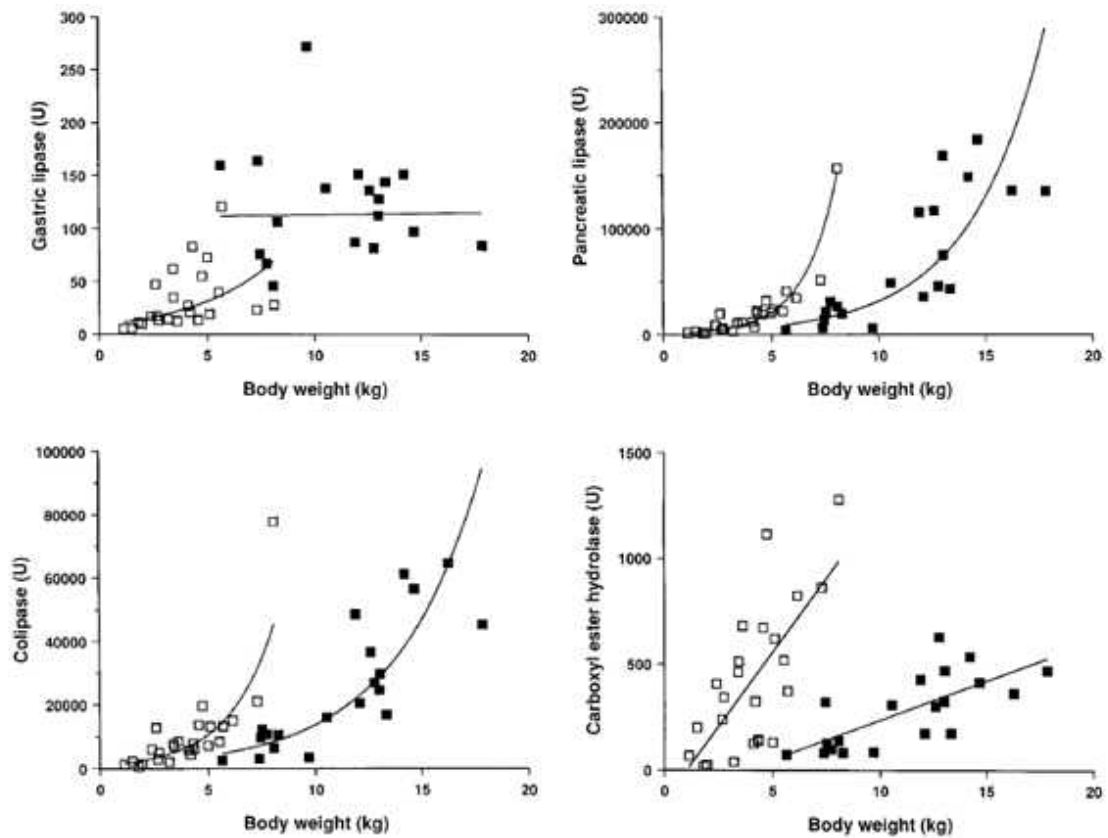


Figure 2. The activity of gastric lipase, pancreatic lipase, colipase, and carboxyl ester hydrolase in relation to the body weight of pigs pre-weaning (□) and post-weaning(■).

The pigs were weaned at 28d of age. The regression lines are shown. Gastric lipase: pre-weaning,  $GL = 10^{.112 \cdot BW + .937}$ ,  $r = .55^{**}$ , post-weaning,  $GL = 10^{.001 \cdot BW + 2.042}$ ,  $r = .02$ . Pancreatic lipase : pre-weaning,  $PL = 10^{.227 \cdot BW + 2.94}$ ,  $r = .93^{***}$ , post-weaning,  $PL = 10^{.123 \cdot BW + 3.27}$ ,  $r = .87^{***}$ . Colipase : pre-weaning,  $CL = 10^{.205 \cdot 205 \cdot BW + 3.00}$ ,  $r = .89^{***}$ , post-weaning,  $CL = 10^{.107 \cdot BW + 3.07}$ ,  $r = .88^{***}$ . Carboxyl ester hydrolase: pre-weaning,  $DEH = 139 \cdot BW - 143$ ,  $r = .71^{***}$ , post-weaning,  $CEH = 37 \cdot BW - 136$ ,  $r = .72^{***}$ .  $**P < .01$ .  $***P < .001$

(Jensen et al., 1997)

Figure 2. showed the activity of gastric lipase, pancreatic lipase, colipase, and carboxyl ester hydrolase in relation to the body weight of pigs preweaning and post-weaning periods (Jensen et al., 1997). The lack of higher lipase levels initially post-weaning pigs fed and oil-supplemented diet might be associated with limited dietary fat digestion and limited growth responses(Cera et al., 1990b). So, many researches focused on how to increase digestibility during the post-weaning period. Processing and supplementation of additive were very common methods to increase feed digestibility.

### **3. Methods to increase feed digestibility**

#### **3.1 Processing in feed mill**

The processing of grains for use as animal feeds, by simple techniques such as grinding or rolling, has been common practice for reducing particle size. Reducing particle size of feedstuff was well known methods to improve feed digestibility in feed industry. However, fine grinding increased the energy costs for processing in feed mill. So many previous researches focused on finding optimum particle size for eliminate disadvantage effects of fine grinding. According to the Healy et al. (1994) only one particle size was to be used for the entire nursery phase, corn milled to approximately 500 $\mu$ m would optimize efficiency of grain in nursery pigs fed. And also pelleting was a process that eliminated bridging problems for diets with small particle sizes, decreased dustiness and segregation of ingredients, and increased bulk density (Skoch et al., 1983).

Table 2. Effects of grain type and particle size on performance of pigs and digestibility of nutrients<sup>a</sup>

Item	Corn, $\mu\text{m}$			
	900	700	500	300
Days 0 to 14				
ADG, g	276	290	332	350
ADFI, g	346	357	354	375
Gain/Feed, g/kg	798	812	938	933
Days 14 to 35				
ADG, g	528	486	508	469
ADFI, g	840	757	791	760
Gain/Feed, g/kg	629	642	642	617
Days 0 to 35				
ADG, g	427	408	438	421
ADFI, g	642	597	616	606
Gain/Feed, g/kg	665	683	711	695
Apparent digestibility (d 39), % <sup>b</sup>				
N	85.1	88.3	89.0	87.6
DM	89.4	90.4	90.8	90.8
GE	89.2	90.6	91.0	91.1

<sup>a</sup>A total of pigs were used (five pigs/ pen and four pens/ treatment) with an average initial BW of 5.3 kg.

<sup>b</sup>Feces were collected after a 4-d adjustment to the diets with Cr<sub>2</sub>O<sub>3</sub> added.

(Healy et al., 1994)

More recently, a range of other techniques have become available, and these can be classified into two basic types - hot processes, in which heat was either applied or created during the treatment process, and cold processes, in which the temperature of the grain was not increased significantly. The hot treatment include steam flaking, micronization, hygienization, roasting and hot pelleting. Steam flaking was often carried out on maize by cooking the grain with steam, and then passing it through rollers to produce a thin flake and dried. Flaked maize was considered to be more available to animals and was of slightly higher digestibility than the unprocessed grain (Mc Donald et al., 2011).

Noland et al. (1976) found that extrusion processing improved the

energy and nitrogen digestibility of sorghum. However, effects of extrusion processing of the carbohydrate sources in diets for weaning pigs had variable effects on growth performance because the degree of starch gelatinization were varied by temperature (Hongtrakul et al., 1998).

### **3.2 Ingredients selection based on digestibility**

Selection of different types and amounts of other feed ingredients also should be based on the three primary criteria of quickly reduction diets complexity to lower feed cost, maximizing feed (energy) intake, and physiology of the digestive system (Tokach et al., 2003). Indeed, ingredient selection in addition to cost should be based on factors including nutrient digestibility, amino acid density, lactose concentration, and stimulatory effects on feed intake and growth. Another consideration is how an ingredient or combination of ingredients will react under various feed processing methods. The use of added fat is an example of this latter consideration. Although added fat is not well utilized by the pig as an energy source immediately after weaning, its inclusion is essential if diets containing high levels of milk and other specialty protein sources are to be pelleted.

#### **Energy sources**

Research with high quality lactose sources has demonstrated linear improvements in pig performance as levels of lactose increased in the diet, even through levels as high as 45% (Mahan, 1990). Recently diet used in commercial application fed immediately after weaning contained more modest and economical levels of 15 to 20% lactose. Lactose additions to the diet continue to improve growth performance until approximately 21 to 28 days after weaning, or 10 to 13 kg (Crow et al., 1995). After this time, lactose can be replaced with more economical carbohydrate sources, such as corn

and wheat.

A high quality, edible or feed grade, dried whey and whey permeate can be good lactose source used in starter diets. Recent research with lactose has focused on evaluating less expensive lactose sources to replace. Several lactose sources have emerged including L-lactose, whey permeates and deproteinized whey. In the past, these ingredients value was uncertain that came from unstable heat treatment in processing. However, processors have improved their understanding of the drying conditions of whey byproduct required to maintain a high quality. The quantity of these products has increased due to human demand for whey protein. Recent trials have proven that other lactose sources can replace whey in the diet with two stipulations: 1) the spray drying must be well controlled, and 2) a high quality protein source must be used to replace the whey protein fraction (Nessmith et al., 1997; Touchette et al., 1995).

Recently, other non-lactose carbohydrates have been introduced as replacements for lactose or dried whey. These carbohydrates include dextrose, sucrose, or byproducts of candy manufactures. The research generally showed that these sources could alter a portion of the whey in the diet, but they should not replace the entire lactose fraction (Stephas and Miller, 1998).

The original high nutrient density diets for weaning pigs contained 8 to 10% supplemental fat to provide levels similar to that of sows milk (Nelssen, 1986). The chain length of a fatty acid is an important determinant of fat digestion and absorption, since different chain of fatty acids have different metabolic routes (Gu et al., 2003). It is well known that short and medium chain fatty acids can absorbed more easier than long chain fatty acids because these fatty acids can bind to albumin directly in blood and be transported to liver.

Glycerol and short chain fatty acids are more soluble in water than long chain fatty acids, thus they can diffuse directly into the enterocyte. The

high digestibility of the medium-chain fatty acids, the higher apparent fat absorption and improved N utilization (percent N retention, lower serum urea) all reflect the beneficial response to dietary coconut oil (Cera, et al., 1989).

The explanation for MCFAs being absorbed faster than LCFA are; (1) esterification of MCFAs is low and most MCFAs can be directly absorbed without hydrolysis by lipase; (2) MCFAs enter the liver directly and rapidly via the portal vein, whereas LCFAs first enter the blood and then into a variety of tissues via the lymph system; (3) glycerides composed of MCFAs can be hydrolyzed faster and more completely and have a higher digestibility and faster clearing from the circulation and (4) MCFAs can be dispersed relatively easily in water and the ratio of re-esterification into chyle particles is low, thus MCFAs and their glycerides are mostly dispersed in intestinal solution and cell membraned and can be absorbed without particle formation (Odle, 1998).

### **Protein sources**

The protein requirement of the weaner pig has to be considered somewhat differently. Raw materials used in diets designed for this age range supply not only nitrogen and essential amino acids but also other proteins that have a secondary role not related to the supply of nutrients. Immunoglobulins, inclusion in feed is more to do with their functional properties than their direct nutritional values be included in this category (Cole and Spent, 2001). This practice involved the use of specialized diets that contained highly digestible ingredients, such as dried whey, spray-dried animal plasma and blood meal, and select menhaden fish meal (Tokach et al., 1994).

From weaning onwards the inclusion of high quality proteins such as fish meal, milk powders and porcine plasma protein gradually reduces as the level of soybean meal and other cheaper vegetable proteins increases (Cole and Spent, 2001). Some vegetable proteins have trigger allergic reactions in



animals and anti-nutritional factor exist in component. Sometimes cheaper vegetable protein source can cause of low palatability in weaning pigs.

Products such as spray-dried porcine plasma (SDPP), where used generally enhance performance through increased feed intake and feed efficiency in the immediate post-weaning period (de Lange et al., 2010). Increasing the level of animal plasma from 5 to 15% in the diet for weaning pigs resulted in a linear increase in pig performance (Dritz et al., 1994). Plasma contains high levels of cysteine, but low methionine levels, making it necessary to formulate for methionine in addition to total sulfur amino acids.

Processed soy proteins decrease transient hypersensitivity and increase villus height, nutrient digestibility, and growth performance as compared to pigs fed diets containing high levels of soybean meal. Friesen et al.(1993) has suggested that moist extrusion of soybean meal greatly improves its nutritive value for weaning pigs. Moist extrusion could be an effective means of improving the value of less expensive soy protein products. Once extruded, products like extruded soy protein concentrate are excellent protein sources to use in combination with other proteins in the starter pig diet (Tokach et al., 2003).

Additionally, dried skim milk, spray-dried blood meal of red blood cells, dried porcine solubles and isolate proteins from vegetable would be good protein source for weaning pig's diet. However, diet cost would be expensive more than fat and carbohydrate sources.

Nutritionist should consider cost effect and specific functions of each ingredients in weaning pig diets. As previously described, selection of ingredients based on its digestibility is the most basic step for increase the performance of weaning pigs. Additionally, some additive which has specific function to increase digestibility in weaning pigs can help to more efficiently use of base ingredients in diets.

### **3.3 Feed additives in weaned pig**

A lot of feed additives have been evaluated for following purposes; (1) enhancing the pigs' immune response (e.g. immunoglobulin;  $\omega$ -3 fatty acids, yeast derived  $\beta$ -glucans), (2) reducing pathogen load in the pig's gut (e.g. organic and inorganic acids, high levels of zinc oxide, essential oils, herbs and spices, some types of prebiotics, bacteriophages, anti-microbial peptides), (3) stimulate establishment of beneficial gut microbes (probiotics and some types of prebiotics), and (4) stimulate digestive function (e.g. butyric acid, gluconic acid, lactic acid, glutamine, threonine, cysteine, and nucleotides) (de Lange et al., 2010).

Many types of feed additives (e.g. enzymes, acidifiers, probiotics, prebiotics, nucleotides and plant extracts) are commercially used in animal feed production for those purposes. Among them, enzymes and acidifiers are good sources for increasing nutrient digestibility.

#### **Enzymes**

The main objective for using exogenous feed enzymes in weaning pig diets has been to improve the nutritive value of feedstuffs. This is achieved through several mechanisms including the breakdown of anti-nutritional factors present in feed ingredients, elimination of nutrient encapsulation effect thus increasing availability, breakdown of specific chemical bonds in raw materials that are otherwise not cleaved by endogenous enzymes, thus releasing more nutrients, and complementation of the enzymes produced by young animals (Simon, 1998; Bedford and Schulze, 1998). Majority of the vegetable feedstuffs used in weaning pig diets contained a considerable amount of non-starch polysaccharides (NSP) whose anti-nutritional effects are well-established and has been a subject of intense research (de Lange et al., 2000). Thus, the use of carbohydrase enzymes in weaning pig diets has

mainly focused on eliminating the anti-nutritional activities associated with the NSP components of feed. Indeed, several studies have shown that with appropriate enzyme preparations, these anti-nutritional effects can be minimized with a potential improvement in the nutritional value of feedstuffs for young pigs (Li et al., 1996) and that a combination of different enzyme activities is required for degradation of complex NSP to improve nutrient utilization (Meng et al., 2005).

In recent studies utilizing an in-situ model of secretory diarrhea in piglets, Kiarie et al. (2008) reported that NSP hydrolysis products generated by incubating soybean meal and canola meal with a multi-carbohydrase enzyme blend were beneficial in maintaining intestinal barrier function during enterotoxigenic *E. coli* infection. Similar observations were obtained with NSP hydrolysis products from wheat and flaxseed. These observations could be explained by various mechanisms, including the possibility that hydrolysis products interfere with the attachment of pathogens to the intestinal mucosa, which is an important step in infection. These products may also act as prebiotics (Cummings and MacFarlane, 2002), favouring the proliferation of lactic acid-producing bacteria as has been shown by Högberg and Lindberg (2004) and Kiarie et al. (2007) and which in turn may indirectly prohibit the growth of certain pathogenic species (Choi et al., 1994).

Feed enzymes may also improve gut health by reducing the intestinal viscosity due to soluble NSP, which might reduce rate of digesta passage, diffusion of digestive enzymes, and increase endogenous gut protein secretions. This will in turn increase substrate availability in the lower gut for microbial proliferation (Verstegen and Williams, 2002). Among other effects, increased viscosity of intestinal digesta in weaned pigs enhanced proliferation of pathogenic bacteria like enterotoxigenic *E. coli* and *Brachyspira pilosicoli* (Mc Donald et al., 2001).

### **Organic and inorganic acids**

Various extensive reviews have been written about the use of organic and inorganic acids in pig diets (Partanen, 2001). The positive effects of feeding acids to pigs on gut health and development, and indirectly on pig health and productivity, may be attributed to various factors, including: (1) anti-microbial activity of non-dissociated organic acids; (2) lowering digesta pH, in particular in the stomach, aiding protein digestion; (3) lowering stomach emptying rate; (4) stimulating (pancreatic) enzyme production and activity in the small intestine; and (5) providing nutrients that are preferred by intestinal tissue thereby enhancing mucosal integrity and function (de Lange et al., 2010). Because of these beneficial and synergistic effects, different combinations of organic and inorganic acids are widely used in diets for weaning pigs. The effectiveness of feeding acids to pigs will vary with the types and combinations of acid, the animal's state and feed characteristics, in particular the diet's buffering capacity (Mroz et al., 2006).

A relatively recent development is the encapsulation of acids for targeted delivery to different gut segments. Studies, such as those conducted by Piva et al. (2007) have shown that relatively simple encapsulation is effective in delaying absorption of dietary acids and allowing more effective delivery of acids to the distal ileum, caecum and colon of piglets. The latter may also be achieved by feeding acids in the form of specific salts. For example, Canibe et al. (2001) showed that feeding potassium-diformate is effective in raising formic acid levels at the distal ileum of pigs at 28 days after weaning. The latter likely contributes to the observed larger positive effects of feeding potassium-diformate on growth performance of pigs, as compared to other forms of formate (Overland et al., 2000).

Among organic acids and in terms of impact on the animal's

physiology, lactic and butyric acid are of special interest (Mroz et al., 2006). The beneficial effects of lactic acid on pig growth performance have been documented and may be attributed largely to its anti-microbial properties and stimulation of endogenous enzyme production (Mroz et al., 2006). Butyric acid is a preferred energy source for enterocytes and has been shown to be effective in enhancing intestinal cell proliferation (Sakata, 1987; Kien et al., 2007).

However, the beneficial effects of feeding butyric acid, or relatively odorless sodium butyrate, on growth performance of newly-weaned piglets could not be detected (Biagi et al., 2006). This lack of response may be attributed to endogenous fermentative butyric acid production (Sakata, 1987), which may be stimulated by feeding easily fermentable fiber from sources such as inulin or beet pulp to young pigs (Jeurond et al., 2008). Indeed, butyric acid-producing bacteria have been identified in the mucosa-associated microbiota (Richards et al., 2005). It is of interest to note that microbes can use gluconic acid as substrate for butyric acid production. Therefore, gluconic acid needed to explore further as a rather inexpensive source of butyric acid for use in animal nutrition (Biagi et al., 2006).

A potential concern is the development of microbial resistance to acids, which has been defined as the ability to withstand an acid challenge of pH 2.5 or less (Castanie-Cornet et al., 1999). Inducible and acid resistance proteins have been observed in *E. coli* (Sato et al., 2000) and *Salmonella typhimurium* (Bang et al., 2000).

## **4. Exogenous emulsifier in weaning pigs**

### **4.1 Definition and characteristics of emulsifier**

An emulsifier (also known as an emulgent) is a substance that stabilizes an emulsion by increasing its kinetic stability. One class of emulsifiers is known as surface active substances, or surfactants. Oil in water emulsions are common in food.

Food emulsifiers may be thought of as designer molecules because the structure and number of heads and tails may be independently varied. A very useful conceptual tool is hydrophile-lipophile balance(HLB). The number and relative polarity of functional groups in a surface-active molecule determine whether the molecule will be water or oil soluble (or dispersible). This concept has been quantitated by calculation of an HLB value describe a given emulsifier. High HLB values are associated with easy water dispensability. Since conventional practice is to disperse the surfactant into the continuous phase, high HLB emulsifiers are useful for preparing and stabilizing oil-in-water (O/W) emulsions. Low HLB emulsifiers are useful for formulation of water-in-oil (W/O) emulsions, such as margarine. Extreme high or low values are not functional as an emulsifiers since almost all of the molecule will be solubilized in the continuous phase. They would, however, be very useful for full solubilization of another ingredient, such as a flavor oil or vitamin, in the continuous phase. At some intermediate values of HLB, the molecule may not be stable in either phase and will result in high concentration at the interface. The practice of adding surfactant to the continuous phase is known as "Bancroft's Rule".

Surfactants may assemble into organized structures described as mesophases or liquid crystals. These bilayer structures adopt several geometric forms: (1) Lamellar-sheets of bilayers where the hydrophilic groups are

paired. Large amounts of water may be trapped in this mesophase, thereby reducing its concentration in the bulk phase. (2) Hexagonal-two cylindrical types. In Type I, the lipophilic tails are contained inside the cylinder and the hydrophilic groups are on the surface. For Type II, the geometry is reversed, with the lipophilic tails on the outside and hydrophilic groups inside the cylinder. (3) Vesicles (liposomes)-Spherical bilayer structures (Hasenhuettl et al., 2008).

#### **4.2 Fat digestion and absorption in stomach and small intestine**

Dietary fat is hydrolyzed in the gastrointestinal tract by lipolytic enzymes secreted from the stomach or pancreas. Gastric lipase is produced in gastric mucosa, and pancreatic lipase is secreted from acinar cells of the pancreas. The total lipase activity in stomach tissue with an optimum pH of 6.2 is only about 3% of that found in the pancreas, although 25–50% of dietary lipid in newborn pigs could be hydrolyzed in the stomach to diacylglycerols, monoacylglycerols and free fatty acids (FFA; Newport and Howarth, 1985; Chiang et al., 1989). Liu et al. (2001) also found that the development of the specific activity of gastric lipase slowed before nursing piglets reached 3 weeks of age, but the total activity of gastric lipase at day 28 was significantly higher than that at day 21. However, the specific activity and total activity of gastric lipase were lowered than those of pancreatic lipase. Thus, the majority of lipid digestion occurs in the small intestine. The presence of fat in the small intestine stimulates the release of the gastrointestinal hormone cholecystokinin (CCK) which stimulates the gallbladder to release bile into the small intestine. The emulsification action of bile salt breaks fats up into small particles so that pancreatic lipase can break down the triacylglycerols into free fatty acids and mono- and diacylglycerols (Figure 3). Pancreatic lipase level is very low until the piglet

receives nourishment by suckling.

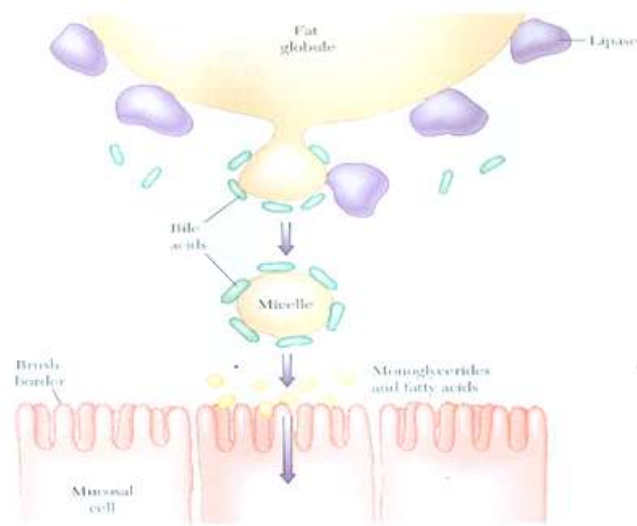


Figure 3. Fat emulsification in small intestine

(Smolin and Grosvenor, 2007)

Once the piglet sucks, pancreatic lipase undergoes a large relative increase, especially from 2 to 4 weeks of age (Cranwell, 1995; Liu et al., 2001). Corring et al. (1978) studied pancreatic digestive enzyme activity in the piglet from 0 to 8 weeks of age and found that activity increased as the piglet grew. Cera et al. (1990) found that pancreatic lipase activity in suckling piglets rose significantly from day 2 to 35 and that weaning at day 21 caused its decrease to a minimum 3 days post-weaning and then rise linearly. However, weaning at day 21 or 35 had no significant effect on lipase activity of the small intestinal mucosa, which rose linearly post-weaning.

It was also found that supplementation with maize oil in the diet had no effect on lipase activities of the pancreas or small intestinal mucosa. Since lipids are insoluble in the aqueous environment of the body they must be modified in order to be absorbed. Mixed micelles are created with bile salts on the outside and monoacylglycerols and fatty acids on the inside. These are



transported to the intestinal cell (enterocyte) wall and absorbed.

Lipids are repackaged in the enterocyte into compounds called lipoproteins - a mixture of lipid components and protein. Phospholipids and protein form the outside layer (more water soluble) and the long-chain fatty acids (LCFA) and cholesterol are on the inside. Chylomicrons are the major lipoprotein made in the intestine to transport dietary triacylglycerols to the cells for energy use. Chylomicrons are absorbed into the lymph system for transport to the cells. Short and medium-chain fatty acids (MCFA) and glycerol are absorbed directly with binding to albumin into the portal vein for transport. It is clear that lipase activity in pancreas and intestinal mucosa is low from birth to 1 week of age or about 1 week post-weaning, which may be a limiting factor affecting fat digestion.

#### **4.3. Functions of bile salts in fat digestibility**

Bile salts are required for supporting the activity of pancreatic lipase as well as for maintaining the polar products of fat hydrolysis in solution. While in the lumen of the small intestine, a fraction of the bile salts is modified by the bacteria present. If the taurine or glycine moieties are removed by microbial enzymes, they are replaced in the liver after reabsorption of the bile salts in the distal ileum.

Cholate precipitates under mild acidic conditions, destroying its detergent effect. However, the protonated amino groups of glyco- and taurocholate maintain the solubility of these bile salts in acidic environments. The highly charged sulfonic acid group of taurocholate further enhances its hydrophilicity and maintains its solubility even at pH 1.0. A biological difference has been observed between glyco- and taurocholate. Some glycocholate appears to be absorbed by diffusion at any point in the small intestine, however taurocholate is highly dependent on the bile salts transport

system in the distal ileum. The peptide link of conjugated bile salts resists hydrolysis by human proteases and peptidases, but is cleaved readily by enzymes produced by the gut microflora.

### ***Enterohepatic circulation***

Bile salts, after being used to support the migration of polar lipids in mixed micelles to the enterocyte, may diffuse back to liver on the form of acids and salts for reuse. Eventually, however, they travel down the small intestine to the far end of the ileum called the distal ileum. Here the bile salts are absorbed into bloodstream and travel through the portal vein to the liver. Absorption in the ileum is via the Na<sup>+</sup>/bile salts co-transporter (Oelkers et al.,1997). Sodium ions occur at higher concentrations in the gut lumen than in the enterocyte, and it is this gradient that drives the bile salts through the apical membrane of the enterocyte, and into the enterocyte. The bile salts in the bloodstream are absorbed by the liver, and the liver directs them back to the gall bladder for storage and eventual release back into the duodenum. This recycling of bile salts is called enterohepatic circulation. The total quantity of bile salts in the human body is 2-4g, but the equivalent of 20-30g enters and leaves the small intestine each day via enterohepatic circulation, that is each bile salt molecule enters and leaves the lumen of the gut about 10 times each day (Brody, 1999).

### ***Deficiency of Bile salts***

Without bile salts, fatty acid absorption is depressed to the same extent as that of triglyceride but even so up to 50% of fat can be absorbed in the absence of bile. On the other hand, other lipid-soluble substances, such as cholesterol, vitamin D, vitamin K, and carotene, are nearly completely dependent on the action of bile to facilitate their absorption. It seems

probably that micellar dispersion of steroids is obligatory for their absorption while, although the absorption of digestion products of triglycerides is facilitated, it is not obligatory. This results in the impaired absorption of vitamins D and K in obstructive jaundice which is often quite out of proportion to the degree of steatorrhea, whereas in pancreatic disease, although steatorrhea may be gross, osteomalacia and a bleeding tendency are extremely rare. It should be noted that intraluminal bile salt deficiency may occur not only in biliary obstruction and with biliary fistulae but also after excision of the ileum and caecum.

#### 4.4 Effects of lecithin/ lysolecithin on growth and digestibility

##### *Lecithin*

Lecithin can easily be extracted chemically (using hexane) or mechanically from readily available sources such as soybeans. Lecithins are composed of phosphoric acid, cholines, esters of glycerol, and two fatty acids; the chain length, position, and degree of unsaturation of these fatty acids vary, and this variation results in different lecithins with different biological functions.

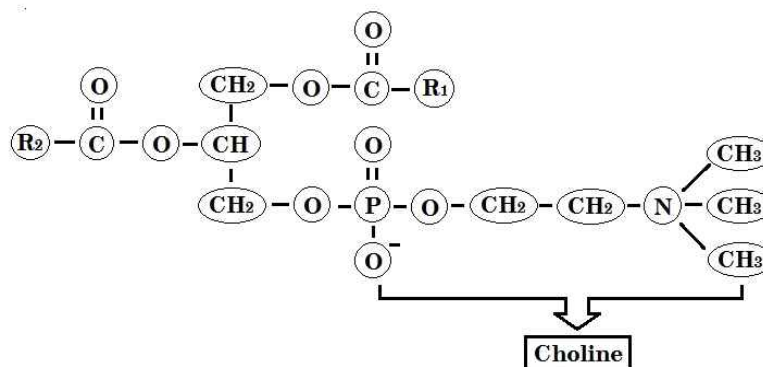


Figure 4. Chemical structure of lecithin

In aqueous solution, its phospholipids can form either liposomes, bilayer sheets, micelles, or lamellar structures, depending on hydration and temperature. It has potential use as an exogenous emulsifier to enhance the utilization of dietary fat. Lecithin increased the apparent digestibility of dietary fat in diets fed to calves (Hopkins et al., 1959), chicks (Polin, 1980), and humans (Aldersberg and Sobotka, 1943). In addition, lecithin can serve as a highly digestible energy source in animal feeds by itself. And its components of fatty acids are very similar with soybean oil (Jones et al., 1992). Research investigating the effect of lecithin on performance of young pigs however its effect is inconsistent and limited (Frobish et al., 1969; Kanyo et al., 1985; Van Wormer and Pollman, 1985; Jones et al., 1990; Jones et al., 1992). While some authors indicated a potential benefit (Jones et al., 1992), others report no enhancing effect on digestibility (Overland et al., 1993, 1995). This discrepancy is most likely attributable to differences in diet characteristics (mostly fat composition) including variations in lecithin content.

Increasing fat digestibility in pigs has been observed related to the inclusion of lecithin in diets containing tallow (Jones et al., 1992; Resis de Souza et al., 1995). This beneficial effect may stem from the highly saturated fatty acid composition of tallow since fat digestibility to a large extent depends on the concentration and proportion of saturated fatty acids (Powles et al., 1994). Lecithin enhances the apparent digestibility of unsaturated fatty acids to a greater extent than that of saturated fatty acids (Soares, 2001). Even though previous lecithin researches show inconsistent results as emulsifier or alternative energy source, it is very important subject of the study which related with emulsifier effects on weaning pig as start point.

### *Lysophosphatidylcholine (LPC)*

In hydrolysed lecithins, a portion of the phospholipids have one fatty acid removed by phospholipase, such phospholipids are called lysophospholipids.

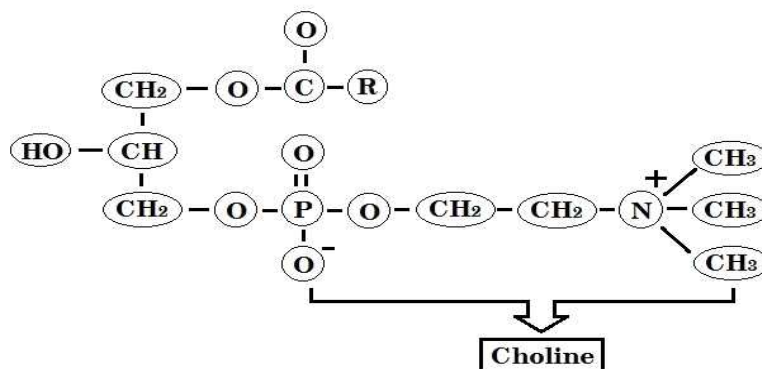


Figure 5. Chemical structure of lysolecithin

The most commonly used phospholipase is phospholipase A<sub>2</sub>, which removes the fatty acid at the C2 position of glycerol. Lysophosphatidylcholine(LPC) can spontaneously form very small micelles because it has a critical micelle concentration (CMC) of 0.02–0.2 mM/L, which is 20–200 times more effective than bile (CMC = 4 mM/ L) and lecithin (CMC = 0.3–2 mM/ L) (Zubay, 1983; Langmuir, 2002). This indicates that LPC has a higher emulsification and micelle formation capacity than that of bile salts or lecithin, thus making it a better source as an exogenous emulsifier. Several researches demonstrated that lysolecithin increased the growth performance when weaning pigs were fed LPC contained diet(Rodas, 1995; Danek et al., 2005) and apparent digestibility of dietary fat was also increased in pigs(Danek et al., 2005). The feeding of lysolecithin tended to lower (P<0.1) serum triglycerides compared with the feeding of the without lysolecithin (Rodas, 1995; Jones et al.,1992). According to the Jones et al.(1992) lower serum triglycerides in pigs fed lecithin may have been due to faster rates of absorption and metabolism of

ingested fat.

#### **4.5 Sodium stearyl-2-lactylate as an emulsifier**

Sodium stearyl-2-lactylate (sodium stearyl lactylate or SSL) is a versatile form of emulsifier and it was approved by FDA recently as a food additive. Commercial grade SSL is a mixture of sodium salts of stearyl lactic acids and minor proportions of other sodium salts of related acids. The hydrophile-lipophile balance(HLB) for SSL is 10-12, thus SSL is slightly hygroscopic, soluble in ethanol, hot oil or fat, and dispersible in warm water. These properties are the reason that SSL is an excellent emulsifier for fat-in-water emulsions and can also function as a humectant. Nevertheless SSL functions very efficiently as an emulsifier, few studies have test in animal feed industry. According to the Jeong et al., (2009) there was no effect of supplementation of the SSL in concentrates on growth rate, feed efficiency and shrinkage in Hanwoo steers during the short final fattening period. Backfat thickness of Hanwoo tended to be thicker when SSL contained diet was provided. Although the functions of SSL was elucidated in ruminant, its effect of SSL in monogastric animals such as pig and poultry was not evaluated. Consequently the experiment was conduct to investigate the supplementation of dietary SSL in weaning pigs. Honestly it was known that fat digestibility in weaning pig showed relatively lower due to the fact that endogenous lipase secretion as well as efficacy are not enough particularly just after weaning.

### III. Efficiency of Sodium stearyl-2-lactate as an Exogenous Emulsifier Supplementation on Growth Performance, Nutrient Digestibility and Blood Component in Weaning Pigs

**Abstract;** An experiment was conducted to investigate the efficiency of sodium stearyl-2-lactate as an emulsifier on growth performance and nutrient digestibility and blood component in weaning pigs. A total of 128 crossbred ([Yorkshire × Landrace] × Duroc) weaning pigs, averaging  $7.07 \pm 1.52$  kg initial body weight were randomly assigned based on sex and initial body weight according to randomized complete block(RCB) design in 8 replicates with 4 pigs per pen. The 2 x 2 factorial design was used in this experiment and the first factor was supplementation level of fat (no fat added or 2% fat added diet), and the second factor was the emulsifier (diets containing 0 or 0.1% of SSL). Experiment was conducted with corn-soybean meal based diet and two phase feeding programs were used. Phase I diet was provided during the first 2 weeks and phase II diet was given for the last 3 weeks. In phase I (0~2 weeks), no significant improvement of body weight and G/F ratio was observed by dietary treatments. In phase II (3~5 weeks), significant interactions were not observed by emulsifier, fat level and emulsifier × fat level in BW, ADG, ADFI and G:F ratio. However, ADFI tended to increase in no added fat treatments (P=0.09) and G:F ratio to increase when dietary emulsifier was supplemented (P=0.08). It is noted that added fat in weaning pig's diet

resulted in decreasing of ADFI however, feed efficiency was improved by inclusion of dietary emulsifier. In digestibility study, crude fat digestibility tended to increase approximately 5% in emulsifier treatments compared to 5% non-emulsified supplemented treatments (P=0.1). This experiment demonstrated that dietary SSL supplementation in weaning pig's diet improved fat digestibility. Moreover HDL:LDL ratio was changed positively when dietary SSL was provided. Consequently dietary sodium stearoyl-2-lactilate(SSL) can be supplemented for improving fat bioavailability in weaning pigs.

**Key words :** Weaning pig, Emulsifier, SSL, Sodium stearoyl -2- lactilate, Growth performance, Nutrient digestibility



## **Introduction**

The world market price for major feed ingredients has increased over the last decades. It is mainly due to expansion of bio-fuel industry for ethanol production which resulted in increasing the cost of feed. This situation had forced the pig industry to find the ways of improving feed efficiency and profitability. Feed cost is approximately 60 -70% of the total cost of animal production, and energy alone contributes about 70% of the feed cost in swine industry (Saleh et al., 2004). This meant that animal feed industry needed to find a way to maximize nutrients bioavailability for minimizing feed cost, and function of exogenous emulsifier (SSL) was evaluated in weaning pig's diet.

Fat supplementation of diets is recognized as efficient method for meeting the high energy requirements of the weaning pigs. However, dietary fat enrichment is difficult in postweaning pigs' diet because of digestive limitations such as lack of enzyme secretion or enzyme efficacy (Cera et al., 1988; Li et al., 1990). However sow' milk fat contained a large amount of short chain fatty acids subsequently it is easily consumable and absorbable (Palmquist et al., 1993). As supplemented fat is comprised mainly long chain fatty acids consequently dietary fat would be absorbed very efficiently when exogenous emulsifier was supplemented particularly in the early of postweaning.

Sodium stearyl-2-lactylate(SSL) is O/W (oil in water) type emulsifying agent which is readily dissolved in water and allows fat to be readily involved in the aqueous phase in the body. For piglet's feed consumption, an emulsifying agent should be well dispersed on oil, because metabolism of digestive organs in the body is generally occurred in hydrophilic condition. And it helps bile salt in the body of animals to convert fat present in the

feed into smaller fat droplets, minimizing the size of micelles before absorption subsequently improving fat absorption efficiently. However, only a few studies were conducted and to make matter worse, researches are focused primarily on lecithin as an emulsifier(W/O; water in oil) which resulted in contrary responses of weaning pig's growth performance (Frobish et al., 1969; Kanyo et al., 1985; Van Wormer and Pollman, 1985; Jones et al., 1990).

Consequently the aim of present research was to evaluate the effects of SSL as an emulsifier agent on nutrient digestibility, blood analysis and growth performance in weaning pigs.

## **Materials and methods**

### ***Experimental design and diet***

A total of 128 crossbred ([Yorkshire × Landrace] × Duroc) pigs, averaging  $7.07 \pm 1.52$  kg initial body weight, were randomly assigned based on sex and initial body weight according to randomized complete block design in 8 replicates with 4 pigs per pen. The 2 x 2 factorial design was used in this experiment and the first factor was dietary fat (no fat added or 2% fat added diet), and the second factor was emulsifier (diets containing 0 or 0.1% of SSL). This experiment was conducted with corn-soybean meal based diet and two phase feeding programs were used. Phase I diet was provided during the first 2 weeks and phase II diet was given for the last 3 weeks. Phase I diet contained 23.7 % crude protein and 1.76 % lysine respectively, was supplied for first 2 weeks. Phase II diet contained 20.9 % crude protein and 1.30 % lysine respectively, was supplied for last 3 weeks. Experimental diets were provided by a local company (Cargill Agri Purina, Inc.) and dry form of emulsifier (Patent, 10-2009-0025752; KIMIN Inc. Seoul, Republic of Korea) was supplemented in basal diet according to each treatments. All nutrients of experimental diets were met or exceeded the nutrient requirement of NRC (1998). Formula and chemical composition of basal diet were presented in Tables 1 and 2.

### ***Animal management and measurement***

Pigs were housed in a half slatted 0.9 x 2.4 m<sup>2</sup> concrete floor, equipped with a feeder and a nipple drinker to allow freely access to feed and water during the whole experimental period. The ambient temperature in the weaning pig's house was kept 31 °C during the first 7 days and lowered to 27 °C after 5 weeks trial. Body weight and feed consumption were

recorded at 0, 2 and 5 weeks to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G/F ratio).

### ***Blood sampling***

Blood samples were taken from anterior vena cava of 5 pigs per treatment for measuring PUN (plasma urea nitrogen), triglycerides, total cholesterol, HDL (High density lipoprotein), and LDL (Low density lipoprotein) when the body weights were recorded. Collected blood samples were quickly centrifuged for 15 min at 3,000 rpm on 4 °C (Eppendorf centrifuge 5810R, Germany). The sera were carefully transferred to 1.5 ml plastic tubes and stored at -20 °C until analysis. To evaluate the efficiency of protein utilization in the body, total PUN concentration was analyzed using a blood analyzer (Ciba-Corning model, Express Plus, Ciba Corning Diagnostics Co.).

### ***Digestibility trial***

Digestibility trial was conducted to evaluate the nutrient digestibility and nitrogen retention in completely randomized design (CRD) with 4 replicates. A total of 20 crossbred barrows ([Yorkshire × Landrace] × Duroc) weaned at  $20 \pm 2$  days of age and weight of  $17.25 \pm 1.14$  kg, were individually allotted to each treatment and housed in a metabolic crate to collect feces and urine separately. A total of 240 g of phase II diet was provided daily. After a 5 days adaptation period, 0.5 % of chromium oxide was manually mixed into the first meal on d 6 as an initial marker. On d 11, 0.5% ferric oxide was used as a finish marker. Fecal and urine collections were initiated for each period when the chromium oxide appeared in the feces and continued until the next appearance of ferric oxide in the feces. Excreta and urine were collected daily and stored -20 °C until analysis.

Collected excreta were pooled, sealed in plastic bags, dried in an air-forced drying oven at 60 °C for 72 h, and ground into 1 mm particles in a Wiley mill for chemical analysis include moisture, protein, fat and ash contents. Total urine was collected daily in a plastic container containing 50 ml of 4N H<sub>2</sub>SO<sub>4</sub> to avoid evaporation of nitrogen from urine and frozen during the 5 day collection period for nitrogen retention analyses.

### ***Chemical and statistical analysis***

Analysis of the experimental diets, excreta and urine was conducted according to the methods of the AOAC (1995). A 2 × 2 factorial analysis was used to determine significance and interaction between main effects of each experiment. The pen of each treatment was used as an experimental unit in growth performance, and each pig was regarded as an experimental unit in blood characteristics, digestive trial and pork quality analysis. All data were analyzed using PROC MIXED procedures of SAS. The statistical model included two main effects, level of energy and emulsifier. The PDIFF option of SAS was used for evaluate differences among treatments. A probability value under 0.05 was regarded as significant difference.

## Results and discussion

### *Growth performance*

Table 3. showed the growth performance such as body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and G:F ratio for each treatment throughout the whole experimental period. In phase I (0~2 weeks), no significant improvement of body weight and G:F ratio was observed by fat or emulsifier supplementation and fat level  $\times$  emulsifier interaction was not detected in growth performance. However, ADG and ADFI were increased when dietary fat was not provided ( $P < 0.01$ ). When pigs were fed no added fat diet, higher growth performance was observed compared to 2% added fat diet. In phase II (3~5 weeks), no significant interactions were observed in emulsifier, fat level and emulsifier  $\times$  fat level on BW, ADG, ADFI and G:F ratio. However, there were tendency of fat effect on ADFI ( $P = 0.09$ ) and emulsifier effect on G:F ratio ( $P = 0.08$ ). In overall phase (0~5 weeks), interactions between emulsifier  $\times$  fat level were not observed. However, when pigs were fed no added fat diet, ADG and ADFI were improved significantly ( $P = 0.03$ ).

Researches to investigate the effect of emulsifier on performance of weaning pigs were inconsistent and limited. Jones et al., (1990) found no effect of emulsifier on growth performance of weaning pigs. In contrast, when 4% sunflower emulsifier was added to diets for weaning pigs, there was a substantial increase in gain compared with diets containing 0, 2, and 6% sunflower emulsifier (Kanyo et al., 1985), indicating that the response might be tightly regulated. Van Wormer and Pollman (1985) reported that a low level of emulsifier (1.5 %) resulted in a response in performance similar to that of diets containing 4% choice white grease, suggesting that emulsifier could serve as a highly digestible fat source for young pigs.

These results were in agreement with the reports of Holzgraefe et al., (1986) and Van Wormer and Pollmann (1985), in which growth performance was not increased by addition of emulsifier to diets for weaning pigs.

The possible reasons of results above mentioned could be attributed to three major factors ; 1) the insufficient experimental period 2) dietary energy density of diet and 3) origin of fat sources.

Due to immaturity of intestinal growth in weaned pigs (Eusebio et al., 1965; Frobish et al., 1969; Cera et al., 1988; Li et al., 1990), the utilization of dietary fat was limited. Weaning pigs did not efficiently use added dietary fat as well as pigs 2 to 3 wk after weaning (Tokach et al., 1995). This study also did not show emulsifier effect in early weaning phase. However, feed efficiency tended to increase when emulsifier was provided to pigs in late weaning phase (P=0.08). It meant, when pigs were fed emulsifier more longer period of time, they could show potential possibility to improve fat absorption efficiency in the body with the help of exogenous emulsifier.

Several studies have shown conflicting result between energy density and feed intake of weaning pig's diet. Zhang et al. (1984) and Urynek and Buraczewska (2003) reported no differences in ADFI with increasing energy density. Furthermore, Beaulieu et al. (2006) demonstrated that pigs with *ad libitum* access to feed showed similar overall energy intake (DE intake of 2.34, 2.30, and 2.45 Mcal/d) when pigs were fed diets containing 3.35, 3.50, and 3.65 Mcal/kg, respectively. In contrast, Nam and Aherne (1994) and Smith et al. (1999) observed decreased ADFI as dietary energy increased. The latter observations were in agreement with the present experiment that low energy density diet increased feed intake subsequently increased ADG was observed, however, G:F ratio was not affected by dietary energy level.

Numerous researches suggested that animal fat regardless of sources was

less digestible by the young pig than that of vegetable origin (Eusebio et al., 1965; Frobish et al., 1969; Cera et al., 1988; 1989; Li et al., 1990; Jones et al., 1992). Unsaturated fats have increased ability to partition into the micellar phase (Freeman, 1969) and could be expected to have higher digestibility than long-chain saturated fats (e.g., tallow and lard). Also, Cera et al. (1989) reported that feeding medium-chain triglycerides (e.g., those found in coconut oil) increased serum NEFA compared with feeding long-chain triglyceride and represented that medium-chain fatty acids are not re-esterified in the gut and, thus, are absorbed directly into the portal blood.

### ***Nutrient digestibility***

Table 4. showed the effect of emulsifier on nutrient digestibility. Significant interactions (fat level, emulsifier supplementation and fat × emulsifier) were not observed in both nutrient digestibility and nitrogen retention, but digestibility of crude fat tended to improve in emulsifier added treatment (P=0.10). When pigs were fed emulsifier supplemented diet resulted in 5% more improved crude fat digestibility than those fed non-emulsified supplemented.

These results were in agreement with Jones et al. (1992) who reported that emulsifiers increased fat digestibility of nutrients in the young pigs, but had minimal effect on growth. Freeman (1969) and Borgstrom (1974) also demonstrated that the capacity of bile salt micelles to solubilize long chain saturated fatty acids and sterols was increased in the presence of phospholipids.

### ***Blood analysis***

Table 5. showed the effects of emulsifier supplementation on blood analysis in weaning pigs. There were no significant interactions in total



cholesterol, triglycerides, LDL and HDL cholesterol. However, dietary fat response was observed in PUN as well as an emulsifier effect in HDL : LDL ratio.

In general, PUN may be an easily measured index or phenotypic marker of efficiency of AA use for maintenance and accretion of lean tissue applicable in selection programs. Plasma urea nitrogen concentrations, as well as urinary excretion of urea, have been measured to estimate AA requirements (Brown and Cline, 1974; Coma et al., 1995; Chen et al., 1995). Nitrogen metabolism has been reported to have a rapid response to changes in dietary amino acid concentrations (Brown and Cline, 1974; Fuller et al., 1979). Thus, Wu and Morris, (1998) reported that a low concentration of blood urea may derive from reduced availability of ammonia caused by enhanced protein synthesis and reduced amino acid oxidation. When a limiting amino acid such as lysine was met, low PUN concentration represented maximal utilization of other amino acids as well as lysine in the body. Result in this study, PUN scores were higher ( $P < 0.01$ ) when pigs were fed 2% added fat diet. Different energy source (animal fat vs starch) may affect on this results. Kashyap et al. (2001) observed that dietary carbohydrate was more effective nutrient than fat in enhancing growth and protein accretion in digestive organs, but they also observed the increment in fat deposition, likely due to a limiting amount of AA available for protein synthesis. Thus, excess energy was likely partitioned to lipid reserves. In the current experiment, due to constant AA:ME, pigs consumed similar amounts of ME and AA, allowing for the increase in protein deposition and the decrease in lipid deposition in pigs consuming the low energy diets.

The HDL:LDL ratio is used as an indicator of cholesterol status and risk of coronary heart disease in humans, and an increasing ratio is considered to be more favorable (Castelli et al., 1977). Emulsifier increased

HDL:LDL compared with non emulsified supplementations during the last phase of experiment. This result was in agreement with Jones et al. (1992) who reported that lecithin increased the HDL:LDL ratio. High-density lipoproteins participate in reverse cholesterol transport, removing excess free cholesterol from the peripheral cells and transporting it to the liver for catabolism (Eisenberg, 1984). In addition, according to Hentges et al. (1985), miniature pigs consumed beef tallow fat source resulted in greater plasma cholesterol concentration, decreased LDL-cholesterol concentration. As a result, emulsifier supplementation increased HDL:LDL ratio in weaning pigs.

## Conclusion

In conclusion, there were no statistical differences on growth performance and fat digestibility in supplementation of sodium stearyl-2-lactylate to weaning pig. However, supplementation of emulsifier tended to improve G/F ratio in phase II (3~5weeks) of experiments. It meant that pigs fed emulsifier more longer period of time, they could show potential possibility to improve fat absorption efficiency in the body. And in the digestibility study, digestibility of crude fat was improved approximately 5% more than fed non-emulsified supplemented. The exogenous emulsifier, SSL, in the diet contributes to improve the digestibility of fat in diet. Consequently emulsifier supplementation in diet improved fat digestibility subsequently positive effect on HDL:LDL ratio was observed.

Although, the application of SSL as an emulsifier in weaning pig diet was evaluated for the first time, the result of SSL addition in weaning pig's diet showed the potential possibility in fat digestibility and blood analysis. therefore, various further studies are needed to determine adequate dosage level and different fat sources for investigating effect of SSL as an emulsifier.

Table 1. Formula and chemical composition of phase I .

	0.1% Emulsifier		No emulsifier	
	2% fat added	no fat added	2% fat added	no fat added
<b>Ingredients, %</b>				
Expanded corn	32.52	32.52	32.52	32.52
Soybean meal	24.68	24.68	24.68	24.68
Barley	10.00	10.00	10.00	10.00
Lactose	11.73	11.73	11.73	11.73
Wheat bran	5.00	5.00	5.00	5.00
Plasma protein	4.00	4.00	4.00	4.00
Isolated soy protein	3.00	3.00	3.00	3.00
Fish meal	3.00	3.00	3.00	3.00
MDCP	1.50	1.50	1.50	1.50
Limestone	1.37	1.37	1.37	1.37
Lysine sulfate (51%)	0.62	0.62	0.62	0.62
Vitamin Mix <sup>1</sup>	0.10	0.10	0.10	0.10
Mineral Mix <sup>2</sup>	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Choline-Cl liq. (75%)	0.04	0.04	0.04	0.04
ZnO (78%)	0.25	0.25	0.25	0.25
Animal fat (tallow)	2.00	—	2.00	—
Tapioca starch	—	2.00	—	2.00
Emulsifier	0.10	0.10	—	—
<b>Chemical composition<sup>3</sup></b>				
Total ME, kcal/kg	3265.00	3184.00	3265.00	3184.00
Total crude protein, %	23.70	23.70	23.70	23.70
Total lysine, %	1.76	1.76	1.76	1.76
Total methionine, %	0.36	0.36	0.36	0.36
Total Ca, %	1.04	1.04	1.04	1.04
Total P, %	0.83	0.83	0.83	0.83

<sup>1</sup> Provided the following quantities of vitamins per kg of complete diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 1,600 IU; vitamin E, 32 IU; D-biotin, 64g; riboflavin, 3.2 mg; calcium pantothenic acid, 8 mg; niacin,16 mg; vitamin B<sub>12</sub>, 12g; vitamin K, 2.4 mg.

<sup>2</sup> Provided the following quantities of minerals per kg of complete diet: Se, 0.1 mg; I, 0.3 mg; Mn, 24.8 mg; Cu·SO<sub>4</sub>, 54.1mg; Fe, 127.3 mg; Zn, 84.7 mg; Co, 0.3 mg.

<sup>3</sup> Calculated value

Table 2. Formula and chemical composition of phase II.

	0.1% Emulsifier		No emulsifier	
	2% fat added	no fat added	2% fat added	no fat added
<b>Ingredients, %</b>				
Expanded corn	40.90	40.90	40.90	40.90
Soybean meal	26.72	26.72	26.72	26.72
Barley	10.00	10.00	10.00	10.00
Lactose	7.46	7.46	7.46	7.46
Wheat bran	5.00	5.00	5.00	5.00
Isolated soy protein	3.00	3.00	3.00	3.00
Fish meal	2.00	2.00	2.00	2.00
MDCP	0.86	0.86	0.86	0.86
Limestone	1.30	1.30	1.30	1.30
Lysine sulfate (51%)	0.21	0.21	0.21	0.21
Vitamin Mix <sup>2</sup>	0.10	0.10	0.10	0.10
Mineral Mix <sup>3</sup>	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
ZnO (78%)	0.25	0.25	0.25	0.25
Animal fat (tallow)	2.00	—	2.00	—
Tapioca starch	—	2.00	—	2.00
Emulsifier	0.10	0.10	—	—
<b>Chemical composition</b>				
Total ME, kcal/kg	3265.00	3184.00	3265.00	3184.00
Total crude protein, %	20.90	20.90	20.90	20.90
Total lysine, %	1.30	1.30	1.30	1.30
Total methionine, %	0.34	0.34	0.34	0.34
Total Ca, %	0.86	0.86	0.86	0.86
Total P, %	0.62	0.62	0.62	0.62

<sup>1</sup> Provided the following quantities of vitamins per kg of complete diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 1,600 IU; vitamin E, 32 IU; D-biotin, 64g; riboflavin, 3.2 mg; calcium pantothenic acid, 8 mg; niacin, 16 mg; vitamin B<sub>12</sub>, 12g; vitamin K, 2.4 mg.

<sup>2</sup> Provided the following quantities of minerals per kg of complete diet: Se, 0.1 mg; I, 0.3 mg; Mn, 24.8 mg; Cu·SO<sub>4</sub>, 54.1mg; Fe, 127.3 mg; Zn, 84.7 mg; Co, 0.3 mg.

<sup>3</sup> Calculated value

Table 3. Effects of emulsifier supplementation on growth performance in weaning pigs<sup>1</sup>

Criteria	Emulsifier		No emulsifier		SEM <sup>2</sup>	P-value		
	fat added	no fat added	fat added	no fat added		Emulsifier	Fat	E X F
<b>Body weight<sup>3</sup>, kg</b>								
Initial	7.09	7.08	7.06	7.07	0.19	0.96	0.99	0.98
2 week	10.87	11.40	10.68	11.36	0.27	0.84	0.28	0.89
5 week	22.24	23.92	21.87	22.93	0.44	0.45	0.13	0.73
<b>ADG, g</b>								
0-2 week	270	309	258	307	8.44	0.66	0.01	0.75
3-5 week	542	596	533	551	11.76	0.26	0.13	0.44
0-5 week	406	452	396	429	9.20	0.34	0.03	0.70
<b>ADFI, g</b>								
0-2 week	388	435	381	434	10.22	0.75	0.01	0.78
3-5 week	872	931	887	927	16.20	0.80	0.09	0.95
0-5 week	634	686	634	681	11.93	0.76	0.03	0.93
<b>G/F ratio</b>								
0-2 week	0.695	0.710	0.677	0.706	0.01	0.75	0.39	0.92
3-5 week	0.621	0.640	0.601	0.594	0.01	0.08	0.97	0.20
0-5 week	0.640	0.659	0.624	0.630	0.01	0.84	0.28	0.89

<sup>1</sup> A total of 128 crossbred pigs was fed from average initial body weight  $7.07 \pm 1.52$  kg.

<sup>2</sup> Standard error of the means.

<sup>3</sup> Values are means for eight pens of four pigs per pen.

Table 4. Effects of emulsifier supplementation on nutrient digestibility in weaning pigs

Criteria	Emulsifier		No emulsifier		SEM <sup>1</sup>	P-value		
	fat added	no fat added	fat added	no fat added		Emulsifier	Fat	E X F
<b>Nutrient digestibility, %</b>								
Dry matter	90.17	89.82	90.47	91.02	0.29	0.23	0.87	0.46
Crude protein	89.19	89.33	90.06	89.71	0.38	0.44	0.90	0.77
Crude ash	64.34	63.82	63.65	67.65	1.27	0.56	0.53	0.41
Crude fat	87.00	78.04	84.92	74.83	1.35	0.10	0.01	0.72
<b>Nitrogen retention (g)</b>								
N intake	7.15	7.19	7.32	7.24	—	—	—	—
Fecal N	0.77	0.77	0.73	0.74	0.03	0.08	0.83	0.86
Urinary N	1.98	1.82	2.08	2.00	0.07	0.34	0.38	0.79
N retention <sup>2</sup>	4.39	4.60	4.51	4.	0.07	0.65	0.52	0.50

<sup>1</sup> Standard error of the means.

<sup>2</sup> N retention = N intake (g) - Fecal N (g) - Urinary N (g).

Table 5. Effects of emulsifier supplementation on blood analysis in weaning pigs<sup>1</sup>

Criteria <sup>1</sup>	Emulsifier		No emulsifier		SEM <sup>2</sup>	P-value		
	fat added	no fat added	fat added	no fat added		Emulsifier	Fat	E X F
<b>Tri-glycerides, mg/dL</b>								
Initial	83	83	83	83	—	—	—	—
2 week	34	40	48	36	3.1	0.46	0.64	0.15
5 week	41	34	35	36	1.9	0.57	0.54	0.31
<b>Total cholesterol, mg/dL</b>								
Initial	144	144	144	144	—	—	—	—
2 week	63	75	62	69	2.9	0.55	0.12	0.18
5 week	85	83	82	75	2.1	0.21	0.32	0.51
<b>LDL cholesterol, mg/dL</b>								
Initial	93	93	93	93	—	—	—	—
2 week	34	35	36	36	1.1	0.52	0.75	1.00
5 week	48	46	50	48	1.4	0.48	0.53	0.90
<b>HDL cholesterol, mg/dL</b>								
Initial	41	41	41	41	—	—	—	—
2 week	21	23	18	23	1.4	0.60	0.35	0.65
5 week	34	32	33	29	1.0	0.25	0.14	0.58
<b>HDL : LDL ratio</b>								
Initial	0.44	0.44	0.44	0.44	—	—	—	—
2 week	0.62	0.66	0.51	0.63	0.04	0.38	0.35	0.61
5 week	0.72	0.70	0.65	0.59	0.02	0.03	0.28	0.66
<b>PUN, mg/dL</b>								
Initial	11.4	11.4	11.4	11.4	—	—	—	—
2 week	13	14	16	15	0.78	0.15	0.87	0.39
5 week	14	13	15	12	0.46	0.67	0.01	0.62

<sup>1</sup> Least square means for five pigs/ treatment.

<sup>2</sup> Standard error of the means.



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## V. Summary in Korean

우리나라의 경우 현재 양돈사료 생산을 위한 원료의 90% 이상을 수입에 의존하고 있는데, 최근에 바이오연료 (biofuel)의 생산과 중국의 곡물 소비량이 폭발적으로 증가함에 따라 원료사료 가격이 급격히 상승하여 일반 농가의 사료비에 대한 부담감이 점점 가중되고 있는 실정이다. 이러한 원료사료의 가격 상승은 사료 내 소화율을 높여 전체적인 효율을 증가시키기 위한 연구가 활발하게 진행되는 계기가 되었으며, 이 중에서도 배합 사료의 가격에 가장 중요한 영향을 미치는 요인 중 하나인 에너지 원료의 소화율을 높이기 위한 실험이 지속적으로 수행되고 있는 상황이다. 여러 에너지 소화율을 높일 수 있는 방안 중에서 현재 가장 직접적인 영향을 발휘하고, 현실적인 방안으로 고려되고 있는 사항이 바로 유화제 (emulsifier)의 첨가인데 아직까지는 레시틴 이외의 첨가제에 대하여는 충분한 연구가 이루어지지 않고 있으며 일관된 결과가 보고되지 않고 있다. 본 실험에서는 이러한 배경을 바탕으로 사료내 유화제로서 sodium stearyl-2-lactylate의 첨가가 이유자돈의 성장성적, 영양소 소화율 및 혈액성상에 미치는 영향을 객관적으로 규명해 보았다.

**실험: 사료내 유화제로서 SSL의 첨가가 이유자돈의 성장성적, 영양소 소화율 및 혈액 성상에 미치는 영향**

평균체중  $7.07 \pm 1.52\text{kg}$ 인 128두의 이유자돈([Yorkshire  $\times$  Landrace]  $\times$  Duroc)을 공시하여 RCB design으로 8반복, 돈방당 4두의 돼지를 배치하여 실험이 진행되었다.  $2 \times 2$  factorial design으로 첫 요인으로 두 종류의 다른 지방 첨가수준(2% 지방급여 or 0% 지방급여)을 두 번째 요인으로 각각 다른 유화제 첨가수준(0 or 0.1% of SSL)으로 실험을 진행하였다. 실험에는 옥수수과 대두박을 기본으로 한 실험사료를 사용하였으며, 두 단계 사료급이 프로그램을 적용하였다. 첫 번째 사료 급이단계는 첫 2주간 급여하였으며, 두 번째 사료 급이단계에서는

마지막 3주간 급여하였다. 0~2주동안에는 실험처리에 따른 체중증가 및 G/F ratio에서 통계적 유의차가 나타나지 않았다. 3~5주 동안에는 유화제 첨가 및 지방 첨가수준변화 그리고 유화제 첨가×지방 첨가 수준 변화에 따른 성장성적의 변화에서 통계적 유의차는 나타나지 않았다. 하지만 지방을 첨가하지 않은 처리구에서 ADFI가 증가하는 경향(P=0.09)이 나타났으며, 유화제를 처리한 처리구에서 G:F ratio가 증가하는 경향(P=0.08)이 나타났다. 이유자돈에서 동물성 지방의 추가급여는 ADFI를 떨어뜨리고 유화제의 첨가는 G:F ratio를 개선시킬 수 있는 것으로 보인다. 영양소 소화율 실험에서는 유화제 첨가 처리구가 첨가하지 않은 처리구에 비해 약 5% 가량 지방소화율이 높아지는 경향(P=0.1)이 나타났다. 유화제로서 SSL의 사료내 첨가가 이유자돈의 지방소화율을 증가시키는 것으로 사료된다. 또한 혈액성상 분석에서는 SSL의 첨가가 HDL:LDL ratio에 변화를 가져온 것으로 보인다. 결론적으로 사료내 유화제로서 sodium stearyl-2-latilate의 첨가는 이유자돈에서 지방의 생체이용성을 증가시킬 수 있는 것으로 사료된다.

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