A Dissertation

for the Degree of Doctor of Philosophy

Effects of Dietary Energy and Amino Acid Levels on Physiological Responses and Reproductive Performance in Swine

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Effects of Dietary Energy and Amino Acid Levels on Physiological Responses and Reproductive Performance in Swine

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Overall Summary

Effects of Dietary Energy and Amino Acid Levels on Physiological Responses and Reproductive Performance in Swine

The objectives of these experiments were conducted 1) to investigate the effect of various dietary energy levels of gestating diets in different season on reproductive performance and milk composition of sows, 2) to investigate the effect of dietary methionine sources and supplementation levels on the body conditions of lactating sows, and 3) to investigate the effect of dietary lysine level on growth performance, nutrient digestibility, blood profiles, fecal *E.coli* counts and occurrence of diarrhea in weaning pigs.

Experiment I. Various Energy Levels of Gestating Diets in Different Season on Reproductive Performance and Milk Composition of Sows

This experiment was conducted to evaluate the effect of various dietary energy levels of gestating diets in different season on reproductive performance and milk composition of sows. A total of 36 mixed-parity (average = 4.92, range = 3 to 8) sows (F1, Yorkshire x Landrace; Darby, Korea) with an initial BW of 218.79 ± 4.74 kg were used for a trial and the experiment was designed as a 2 x 4 factorial arrangement of treatments in a split-plot design. The season of mating (summer-July and winter-January) was regarded as main plot and the four different dietary energy levels of diets for gestating sows (3,165, 3,265, 3,365 or 3,465 kcal of ME/kg) was subplot. The experimental diets (Cargill Inc., Korea) containing different energy levels were provided 2.4kg daily in gestation but one diet was provided *ad libitum* in lactation regardless of treatments. The lower weight gain from 0 to 110 days of gestation were shown when sows were fed low energy diet (linear response, $P<0.05$) and sows were mated during winter (season response, $P<0.01$). Both backfat thickness of 110 days of gestation (linear response, $P<0.05$) and backfat gains from 0 to 110 days of gestation (linear response, $P<0.01$) were subsequently shown to be increased when dietary energy level was increased. The changes of backfat thickness during lactation were higher when dietary energy level were increased during gestation (linear response, $P<0.01$). However, the litter weight, piglet weight and piglet weight gain during the whole lactating period were not significantly different among the treatments. In the results of the composition of sow colostrum and milk, feeding diets containing different energy levels and mating sows in different seasons had no detectable effects on the content of fat, lactose, protein and total solid except the ratio of solid not fat at 7 days postpartum (season response, $P<0.05$) and the fatty acid profiles. The myristic acid at 7 ($P<0.05$), 14 ($P<0.01$) and 21 ($P<0.05$) days postpartum, the palmitic acid at 14 days postpartum ($P<0.01$), the palmitoleic acid at 14 days postpartum ($P<0.05$), the stearic acid at 7 ($P<0.01$) and 14 ($P<0.01$) days postpartum and the oleic acid at 7 ($P<0.05$), 14 ($P<0.01$) and 21 ($P<0.05$) days postpartum were higher when sows were mated during summer but palmitoleic acid at 14 days postpartum was lower when dietary energy level was increased during gestation (linear response, $P<0.05$). There was a significant dietary energy level x season interaction in the content of stearic acid at 7 day, resulting in high values when dietary energy level was decreased in sows mated during winter compared with those mated during summer. This experiment demonstrated that reproductive performance of sows was not affected by dietary energy level during gestation when 2.4 kg of diet was provided daily. Consequently constant dietary energy level can be provided to gestating sows regardless of season when environmental temperature in gestating house can be controlled during winter.

Experiment II. Effects of Dietary Methionine Sources and Supplementation Levels on Body Conditions of Lactating Sows

This experiment was done to evaluate the effect of different dietary methionine sources and supplementation levels on body conditions of lactating sows. A total of 35 mixed-parity (average = 4.86, range = 2 to 6) gestating sows (F1, Yorkshire x Landrace; Darby, Korea) with an initial BW of 235.17 ± 4.05 kg were used in a 3-week trial and sows were allotted to 1 of 5 treatments based on BW and backfat thickness with 7 replicates by 1 + 2 x 2 factorial arrangement. The experimental treatments were divided by methionine sources and levels; 1) Control: basal diet (NRC requirement), 2) 0.05% DL-methionine: basal diet + 0.05 % DL-methionine, 3) 0.10% DL-methionine: basal diet + 0.10% DL-methionine, 4) 0.05% L-methionine: basal diet + 0.05% L-methionine, 5) 0.10% L-methionine: basal diet + 0.10% L-methionine. The experimental diets were formulated based on corn and soybean meal and contained 3,265kcal of ME/kg, 16.80% crude protein, 1.08% lysine, 0.20% Na, 0.90% Ca, and 0.70% total P, respectively. All other nutrients were met or exceeded the requirements of NRC (1998). There was no significant difference in body weight and backfat thickness of lactating sows by methionine treatments during lactation. The body weight changes in lactation (0-21 d) were affected by the supplementation level of L-methionine, resulting in linear and quadratic methionine level responses ($P<0.05$), and then the detectable effect by the supplementation of DL-methionine was also shown, resulting in linear methionine level response ($P<0.05$). Although there were no significant differences, the changes (0-21 d of lactation) of backfat thickness showed similar trend numerically with body weight changes, and then the weaning to estrus interval (WEI) was shortened when L-methionine was supplemented to lactating diet (sources response, $P<0.05$). Supplementing of methionine to lactating diets did not show improvements of body weight and weight gain of nursery piglets, and then voluntary feed intake of lactating sows was also not affected by the dietary treatments. The colostrum and milk composition including milk fat, protein, lactose, total solid and solids-not-fat were not affected by dietary treatments and no differences were found on serum methionine level of piglets in 21 d of lactation. However, when sows were fed diets contained 0.10% DL-methionine, 0.05% and 0.10% L-methionine showed higher serum methionine levels compared to those fed control diet ($P<0.01$), resulting in a linear effect by supplementing DL- and L-methionine ($P<0.01$). In methionine level of sow milk, the linear increase was found as dietary L-methionine level increased ($P<0.05$). Consequently, methionine supplementation in lactating diet did not show differences in growth performance of nursery piglets, milk composition and voluntary feed intake of sows regardless of sources and supplementation levels except for body weight changes in gestation and methionine level of serum and milk of lactating sows. The supplementation of L-methionine was more efficient source to reduce body weight loss and to improve the body conditions of lactating sow than that of DL-methionine treatment.

Experiment III. Effect of Dietary Lysine Level on Growth Performance, Nutrient Digestibility, Blood Profiles, Fecal *E.coli* Counts and Occurrence of Diarrhea in Weaning Pigs

A study was conducted to invesetigate the effect of dietary lysine level on physiological responses, nutrient digestibility, blood profiles, fecal *E.coli* counts, and incidence of diarrhea in weaning pigs. A total of 128 piglets [(Yorkshire × Landrace) × Duroc] (BW = 7.24 ± 0.03 kg; weaned at d 28 ± 2) were randomly allocated to 1 of 4 treatments in a randomized complete block (RCB) design and 8 replicates with 4 pigs per pen. Dietary treatments were divided by the dietary level of lysine; 1) N + 0.15% (NRC requirement + 0.15% lysine), 2) N (NRC requirement), 3) N – 0.15% (NRC requirement – 0.15% lysine), 4) N – 0.30% (NRC requirement – 0.30% lysine). The diets contained 3,265 kcal ME/kg and 23.70% crude protein and the other diets contained 3,265 kcal ME/kg and 20.90% crude protein were supplied for the phase I and II, respectively. All other nutrients were met or exceeded requirements of NRC (1998). There were no significant differences in ADG, ADFI and G/F ratio of phase I. However, ADG and G/F ratio at phase II were slightly decreased as dietary lysine level reduced, resulting in linear ($P<0.05$) and quadratic responses ($P<0.05$). Feeding of high lysine diets showed lower BUN concentration (linear responses at 2 and 5 wk and quadratic response at 5 wk, $P<0.01$) and urinary nitrogen (linear response, $P<0.05$), whereas the nutrient digestibilities of dry matter, crude protein, crude ash, and crude fat were not affected by dietary treatments. The piglets fed high lysine diets had higher moisture content of feces at 2 wk ($P<0.05$), fecal *E.coli* counts at 5 wk ($P<0.01$), and incidence of diarrhea ($P<0.05$) compared with those fed low lysine diets with linear responses. Consequently, these results suggested that 11 to 20 kg pigs fed diets containing 20.9% crude protein had a dietary lysine requirement for approximately 1.15% lysine/kg of diet, and piglets fed high lysine diets showed higher nitrogen retention, incidence of diarrhea, and fecal *E.coli* counts relative to those fed low lysine diets.

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List of Abbreviation

ADFI	average daily feed intake
ADG	average daily gain
BFT	backfat thickness
BUN	blood urea nitrogen
CP	crude protein
DE	digestible energy
FTA	free trade agreement
GE	gross energy
GI	gastrointestinal tract
GLM	general linear model
Ig	immunoglobulin
LCT	lower critical temperature
ME	metaboilzable energy
MSY	market pigs per sow per year
NE	net energy
NSP	non-starch polysaccharide
SBM	soybean meal
UCT	upper critical temperature
VFA	volatile fatty acid
WEI	weaning to estrus interval

Chapter I. General Introduction

Corn and soybean meal have been widely used in the swine feed in South Korea. However, increasing biofuel demand to produce ethanol caused high price of corn and soybean meal and greater demand of new feed ingredients in many countries. Then, the outbreak of foot-and-mouth disease occurred in South Korea in 2010-2011, resulting in the culling of more than three million of pigs and severe economic depression of swine industry, and free trade agreement (FTA) between South Korea and other countries (EU, USA and Chile) was associated with decreased price of Korea pork derived from severe economic competition against these countries. Therefore, the strategies for improving feed efficiency and decreasing production cost as well as increasing swine productivity were needed to lead an undisturbed domestic swine industry.

Market pigs per sow per year (MSY) in Korea was approximately 15.1 (Korea pork producers association, 2011), but those in Netherlands, Denmark, France, Belgium, Germany and United Kingdom were 26.0, 25.5, 24.7, 22.6, 22.5 and 21.0, respectively (Pig international, 2011). There are several reasons for low productivity in Korea, such as the excessive levels of dietary nutrients due to worry of animal growth performance, and the application of nutrient requirements and animal management technique without the verification experiment in domestic environment. Then, among these reasons, nutrient requirement is more important than other factors because this reason could be revised easily when the exact nutrient requirement is decided by additional experiments. Although many nutritionists had interest in the effects of dietary nutrients on physiological responses, reproductive performance and immune response of pigs, modern pigs have been improved genetically for higher productivity and efforts to evaluate the exact requirement of some nutrients were insufficient as time passed.

To investigate the energy requirements of gestating and lactating sows, a considerable amount of experiments had been conducted, and the assumptions of several references to calculate those requirements were already submitted (NRC, 1998). However, little information is available for evaluating the consequences of additional energy intake to overcome low environmental temperature in sows, even though the temperature of both in summer and winter is hard to control in Korea because of great temperature differences between these two seasons, often exceeding 50°C. Mount (1972) and Sainsbury (1972) demonstrated that energy requirements of sows could be changed by environmental temperature, wherein maintenance energy to make heat was affected by lower critical temperature (LCT), but the indoor temperature of pig building is controlled by heater in Korea. Therefore, conducting experiment to investigate the response of dietary energy levels on physiological responses and reproductive performance of gestating and lactating sows in domestic environment could be one of the nutritional methods to improve sow productivity.

It is generally well known that bioavailability of DL-methionine relative to L-methionine differed by the species due to different enzymatic activity to convert D-form in L-form, and findings in humans indicate low bioavailability of D-methionine (Stegink et al., 1986; NRC, 2012). In case of swine, many researchers considered that D-form has approximately 100% efficiency relative to L-form except for young pigs (Chung and Baker, 1992), and the experiments on methionine requirement and availability were concentrated with other methionine sources, such as methionine hydroxyl analog (Reifsnyder et al., 1984). However, dietary methionine requirement of many references were changed as genetic traits of pigs were changed, and it may affect the utilization efficiency of DL-methionine because the ability of converting enzyme is limited relative to increased intake of D-methionine. Therefore, this study evaluated the utilization efficiency of DL-methionine relative to L-methionine and optimum requirement of dietary methionine for lactating sows.

The incidence of diarrhea after weaning can be negatively affected by applying excess dietary lysine and protein, because undigested lysine and protein were associated with the proliferation of pathogenic bacteria (Ball and Aherne, 1987). Many swine producers ignored this bacterial fermentation even though toxic substances could be derived from these processes, resulting in decreased feed intake and daily gain (Porter and Kenworthy, 1969; Dong et al., 1996). Therefore, this study investigated the effects of dietary lysine level on physiological responses and other parameters in weaning pigs.

For increasing swine productivity and decreasing feed and production cost at swine farms, three experiments were conducted 1) to investigate the effect of various dietary energy levels of gestating diets in different season on reproductive performance and milk composition of sows, 2) to investigate the effect of dietary methionine sources and supplementation levels on body conditions of lactating sows, and 3) to investigate the effect of dietary lysine level on growth performance, nutrient digestibility, blood profiles, fecal *E.coli* counts, and incidence of diarrhea in weaning pigs.

Chapter II. Literature Review

1. The Strategies for Improving Productivity and Saving Feed Cost

1.1 General Approaches

1.1.1 Nutrient Requirement of Pigs

A number of findings during the past 60 years have examined the effects of dietary level of nutrients on physiological responses and reproductive performance of pigs to make maximum growth rate and to save feed cost (NRC, 2012). In commercial pig production, supplying exact amounts of nutrients are very important to maximize economic efficiency, wherein surplus nutrients are excreted from the body. Among many nutrients, the nutritionists had selected several nutrients, such as energy, amino acids, minerals, vitamins, waters and so on, because these nutrients are essential for the synthesis of muscle, adipose tissue, bone, hair, skin, and other body components and for body maintenance, growth, reproduction and lactation. Although these nutrients play a role respectively in the body, the requirements of protein, amino acids, and energy are most important to maximize economic efficiency, because the supplements of these nutrients have high price and the role of these nutrient are more important than others. Recently, the negative effects of feeding diets containing excess level of nutrients have been presented in many findings, such as increased production of toxic substance, harmful bacterial fermentation, incidence of diarrhea and environmental pollution source (Porter and Kenworthy, 1969; Ball and Aherne, 1987; Dong et al., 1996; Hobbs et al., 1996). Then, modern pigs have been improved genetically for high productivity, growth rate and feed efficiency, but little information is available to evaluate increased or decreased requirement of some nutrients despite of genetic changes. Therefore, in order to improve swine productivity and decrease feed cost, the experiments to evaluate changed requirements of some nutrients have to be conducted, along with those to evaluate the interaction response between dietary treatments and domestic environmental factors.

1.1.2 Feed Ingredients

In general, corn and soybean meal have been widely used as feed ingredients for swine in South Korea. However, biofuel demand lead to increase ethanol production had prompted in high price of corn and demand of new feed ingredients for livestock production in many countries. Many attempted has been done to find new feed ingredients, alternatives to corn and soybean meal with a competitive price, steady supply, and similar growth performance of animals. Among many feed ingredients, palm kernel meal (PKM) and copra meal (CM) have been used frequently relative to other feed ingredients in South Korea. PKM and CM are found in large quantities in a number of tropical countries and are available at competitive prices. These feed ingredients were associated with high fiber, low palatability and lack of several essential amino acids, coupled with some anti-nutritional properties as mannan, galactomannan, xylan and arabinoxylan (Thorne et al., 1990). Mannan is a type of non-starch polysaccharides (NSP) which is mainly composed of mannose, and then it is well known that this nutrient reduces the rate of glucose absorption, nitrogen retention, and fat absorption due to its high viscosity (Rainbird et al., 1984; Nune and Malmlof, 1992; Dale, 1997). The NSPs of CM and PKM are mainly composed of pure mannan and galactomannan, and mono-gastric animals have a lack of enzyme for degrading these nutrients. Thereby, β-mannanase have been supplied with these feed ingredients to improve nutrient utilization through degrading mannan and galactomannan to a low molecule oligosaccharide or monosaccharide like mannose (Sundu et al., 2006). However, the price of CM and PKM was also elevated dramatically due to increased demand of domestic feed companies during the past 10 years. Then, many experiments to replace these feed ingredients have been conducted for several years, and some feed ingredients have been selected by nutritionists, such as rapeseed meals and cassava residue. At this moment, it is shown that more experiments are essential to determine supplementation levels and to find controlling methods of anti-nutritional factors in individual ingredient.

1.1.3 Animal Management Techniques

Many experiments have been carried out to evaluate the effects of pig management techniques on swine productivity. In Korea, creep feeding has been used frequently to improve body condition of lactating sows and growth performance of their progeny, and there are many findings presenting the positive effects of this management technique on pre- and post-weaning performances of piglets (Bruininx et al., 2002; Klindt, 2003; Pluske et al., 2007). However, there were also opposite results that creep feeding had no effects on growth rate of piglets after weaning (Fraser et al., 1994; Sulabo et al., 2010), and the creep feed production had a proportion of 3% approximately in total swine feed production in Korea even though the duration of creep feeding is very short than other phases. Therefore, the additional studies to determine the duration of creep feeding and the response of nursery piglets and lactating sows on this management technique were needed to solve the confusion of farmers.

When the interval between artificial insemination and ovulation is from 0 to 24 hours, fertilization efficiency is optimal (Kemp and Soede, 1997). However, the high variation in duration of estrus induces a high variation in the interval from onset of estrus to ovulation (Steverink et al., 1999), wherein the duration of estrus could be varied from 24 to 96 hours (Weltze et al., 1994). Thereby, determining optimal time of insemination after the onset of estrus was one of the most important factors to improve sow productivity. Kemp et al. (1996) and Soede et al. (1995)

suggested that sows inseminated between 0 and 24 hours before ovulation showed high fertility consistently, and Xue et al. (1998) demonstrated that conception rate, pregnancy rate, and farrowing rate were not differed even though mating frequency was changed. In addition, Weltze et al. (1994) recommended that the interval from onset of estrus to first artificial insemination should be decreased as weaning to estrus interval increased, and Steverink et al. (1999) reported that the best reproductive performances were depended on the time of insemination relative to ovulation, so each farm should determine individual insemination strategy.

Above these strategies, various management techniques have been applied without verification test in domestic environment as well as the methods of tooth clipping, the tools for artificial insemination, separate-sex rearing, dry and liquid feeding, and so on. However, these untested techniques should be considered carefully because these strategies could be reasons of growth check, decreased reproductive performance, and feed efficiency.

1.1.4 Other Strategies

Recently, the carcass lean content of pigs has been increased significantly by intense selection and swine breeding, and the modern sows are bred in the way to improve litter size, lean content and fat content (Dyck and Strain, 1983; Cassar and King, 1992; Edwards et al., 2002). Like this, breeding technology have been used frequently to improve swine productivity and consumer acceptance for pork during the past 60 years, and then there is a greater achievement, wherein the demand for pork and reproductive performance, growth rate and feed efficiency of pigs are highly increased compared with the past. However, a cautious approach is recommended to apply this strategy individually, because a high cost and accumulated breeding technique are essential and long period is needed to create profits. Except for breeding technique, many other strategies have been examined to improve swine productivity and to save production cost, such as the application of prevention disinfection for preventing pathogen infection, automatic management system for decreasing labor costs, purification treatment plant for reducing costs of excrement handling, and so on.

1.2 Nutritional Approaches for Pigs

1.2.1 Feed Intake

Generally, the experiments about controlling feed intake of pigs had been concentrated on sows, because the feed for pigs to produce meat were fed *ad libitum*. Various feeding strategies for gestating sows have been applied to accomplish maximum litter size and body conditions of sows (Boyd et al., 2000; Trottier and Johnston, 2001). However, the findings to evaluate the effects of feeding strategies showed inconsistent response of sows, and mechanisms involved in physiological parameters and reproductive performance were not clearly understood (Piao et al., 2010). Dyck et al. (1980) demonstrated that the gilts fed increased amounts of diets showed decreased survival rate of embryo in d 28 of gestation, and Pharazyn et al. (1991) also reported that restriction feeding of 1.8 to 2.0 kg/d during early gestation saved feed cost without any worry of reproductive performance in gestating sows. However, Hughes (1993) presented that increased feeding level in early gestation had effects on increasing maternal tissue deposition, and the rate of embryo survival was not differed among dietary treatments. In general, it is well known that IGF-1 (insulin like growth factor-1) has influence on cell proliferation, and especially, it can connect to the receptor of theca cell to stimulate the secretion of androgen in ovary of animals (Whitley et al., 1998). Then, the follicular growth can be increased by the action of androgen, and the ovulation of ovum is controlled by this process (Hammond et al., 1998). All hormones are associated with feedback system to prevent excessive secretion, and IGF binding protein inhibits the connection between IGF-1 and theca cell by connecting IGF (David et al., 1997). However, there is a finding reporting that this binding protein also can be connected with insulin (Spicer et al., 1992; David et al., 1997), and it means increased concentration of insulin derived from increased feeding level can decrease the follicular growth by the feedback system of IGF-1 binding protein during early gestation.

Similarly, the findings in middle gestation had inconsistent results, related to embryo development and the growth of mammary gland. Gatford et al. (2003) demonstrated that the sows fed high amounts of diets from 29 to 50 d of gestation showed improved differentiation of muscle fibers of piglets, and Weldon et al. (1991) reported that milk production and the growth of mammary gland could be decreased by increasing feeding levels in the middle of gestation. During the middle of gestation, fetal growth is relatively low, and excess feeding levels can induce poor body condition or backfat depth. Therefore, a considerable approach should be applied to use this strategy in middle of gestation.

Whittemore et al. (1984) showed no detectable effects of increased feeding level during late gestation on litter size, but Kirkwood (1988) and Noblet et al. (1990) demonstrated that the birth weight of piglets was increased by increased feeding levels in late gestation. In addition, Miller (1996) suggested that sow condition after farrowing could be improved by increased feeding level in late gestation, resulting in increased birth weight of piglets and milk production. There were many researches supporting feeding high amounts of diet during late gestation improves feed intake during lactation because physical change of GI tract facilitates the adaptation of the sows to the drastic increase of feed consumption (Weldon et al., 1994; Matte et al., 1994; Vestergaard and Danielsen, 1998; Renteria-Flores et al., 2008). However, the responses were varied by the parity of sows, the number of piglets born alive, dietary energy, and fiber levels, and it is definite that the constant feeding ration is efficient way in the aspect of manpower management. Consequently, additional studies were needed to determine optimal feeding strategies for gestating sows.

1.2.2 Energy

Dietary energy levels had effects on voluntary feed intake, the intake of other nutrients, reproductive performance, feed efficiency, and immunity in pigs, and it was closely associated with the feed cost due to the high price of fat sources. Dyck and Strain (1983) and Cassar and King (1992) demonstrated that the reproductive performance of sows could be improved as dietary energy level was increased, and Amusquivar et al. (2010) reported that diet-derived long chain fatty acids could be taken up by the mammary gland for milk synthesis. However, there are several reports suggesting the negative effects of high energy levels in diet on rate of embryo survival (Liao and Veum, 1994), voluntary feed intake of sows during lactation (Dourmad, 1991) and excess increment of body weight and backfat thickness of sows during gestation (Dourmad et al., 1999). The NRC (1998) recommended 6,015 to 6,395 kcal of ME/day as a nutrient requirement of gestating gilts and sow, and revised energy requirements of NRC (2012) were 6,427 to 6,928 kcal effective ME/kg during below d 90 of gestation 7,775 to 8,182 kcal effective ME/kg during above d 90 of gestation.

In South Korea, increasing feeding level and energy level in domestic feed companies have been frequently used to compensate heat loss during winter season because the temperature of facilities for pigs is hard to control. However, excess energy intake may result in decreased feed intake in lactation, increased body weight and backfat losses in lactation, delayed weaning to estrus interval, and increased feed cost.

1.2.3 Protein and Amino Acids

The feed efficiency is one of the important factors for improving swine productivity, because the feed cost largely depends on this parameter in most swine industry. This is especially true for protein supplements as a major portion of feed costs is controlled by the supplementation level of these ingredients. Moreover, feeding excess level of protein to pigs can increase the amounts of pollution sources as well as excreted nitrogen and incidence of diarrhea in piglets. Therefore, improving utilization efficiency of protein has positive influences on profitability of domestic swine farm and environmental protection.

Protein supplements have different nutritional values individually, because the availability of amino acids and the ratio between the essential amino acids and non-essential amino acids differ from supplement to supplement (Baker, 1997). Then, the ideal pattern and profiles of amino acids is easily variable depending on change of new tissue synthesis, because there are changed amino acid content of pig tissue from birth to market weight (Mahan and Shields, 1998). Kolstad and Vangen (1996) demonstrated that protein turnover is important to determine energy and protein requirement of pigs because it is related to lean growth rate, and NRC (2012) assumed these requirements by a mathematical model derived from several findings. However, modern pigs have better performances of lean growth, fetal growth, litter size, and milk production relative to those references of NRC (2012), and it can induce the problems with physiological responses and reproductive performance in pigs regardless of age. There were many studies suggesting the negative effects of low protein intake in pigs. In sows, decreased birth weight, decreased litter weight gain, increased body weight losses of sows and decreased milk production were detected when dietary protein intake was insufficient (Mahan and Grifo, 1975; Mahan, 1977; Schoknecht et al., 1993). Then, the piglets fed low protein diets showed increased BUN concentration, reduced nitrogen digestibility and growth performance compared with those fed high protein diets (Eggum, 1970; Urynek and Buraczewska, 2003).

1.2.4 Fat and Fiber

Associated with the addition of fat to diet of sows, the effects of energy sources including fat and starch sources have been studied in many findings to decrease feed cost and improve swine productivity. Tilton et al. (1999) demonstrated that addition of fat to the diets of lactating sows have influences on increasing fat content of milk, the growth performance of nursery piglets, and litter weight, with no difference in backfat losses of lactating sows. However, van den Brand et al. (2000) suggested that adding carbohydrate sources had effects on improving milk composition, piglet body composition, voluntary feed intake, and energy balance of sows, whereas adding fat sources had no influences on improving energy balance of sows, and Nelssen et al. (1985) and Kemp et al. (1995) also reported that the lactating sows fed diets containing high level of starch sources showed improved rate of protein catabolization, litter size, energy digestibility, body conditions, litter weight in lactating sows relative to those fed containing high level of fat sources. Usually, the necessary expense for adding fat sources is higher than those for adding carbohydrate sources to increase dietary energy levels, so using carbohydrate sources for controlling dietary energy levels could be efficient way to save feed cost.

Fiber sources have been used for ruminant animals because the rumen microbes could digest fiber fractions, but these ingredients were not used frequently for mono-gastric animals because endogenous digestive enzyme of mono-gastric animals could not digest fiber fractions without exogenous enzymes derived from diet such as mannanase, cellulase, and etc (Noblet and Etienne, 1989; Black et al., 1993). However, there were several findings reporting positive effects of fiber diets on physiological responses and reproductive performance of pigs. Grieshop et al. (2001) demonstrated that increased total born number of piglets was observed when sows were fed high fiber diets, and Lee and Close (1987) also suggested that stereotypic behaviors and physical activity of gestating sows could be decreased by high fiber diets, resulting in improved energy digestibility, embryo development and maternal energy balance. Many domestic swine farms have been applied the restriction feeding method for gestating sows, so high fiber diets could be a strategy to improve swine productivity and to decrease production cost.

1.2.5 Feed Additives

Many feed additives such as probiotics, prebiotics, acidifier, emulsifier, enzymes and plant extracts have been used to improve the growth performance, reproductive performance, feed efficiency, immunity and productivity in swine industry, and the importance of these additives was increased due to the ban of antibiotics in South Korea. The positive effects of probiotics had reported many times, wherein it can help the dominance of beneficial bacteria in GI tract of pigs, resulting in improved growth performance, body conditions of lactating sows, fertility, milk fat and protein contents, and piglet survival rate (Stamati et al., 2006; Kim et al., 2008). Then, prebiotics also have been used frequently because it can provide substrates for beneficial microbes, resulting in increased pre-weaning growth, reduced return to estrus interval and pre-weaning mortality (O'Quinn et al., 2001; Maxwell et al., 2003).

Recently, many new feed ingredients have been applied for swine feed to replace the corn and soybean meal, because the prices of these feed ingredients using mainly in swine feed are highly increased. Then, enzymes to digest fiber fractions have been used frequently to improve the nutrient value of fiber supplements. Bach-Knudsen et al. (1988) reported that high fiber content of diets have contributions to block the access of digestive enzymes to the cell contents, resulting in increased passage rate of digesta and reduced time to digest nutrient as well as reduced nutrient digestibility (O'Doherty and McKeon, 2000). Then, Araki and Kitamikado (1988) presented that these problems could be solved by supplementation of carbohydrate enzymes, such as mannanase and cellulase.

Above these feed additives, acidifier, emulsifier and plant extracts were also used to decrease gut pH of piglets after weaning, to improve fat digestibility of pigs, and to induce anti-oxidant reaction in the body of pigs, respectively. Then, many commercial products of these ingredients are on sale in commercial area.

2. Dietary Energy Levels and Different Seasons in Gestating Sows

2.1 Energy Requirements of Gestating Sows

Metabolizable energy (ME) system had been used frequently during the past 60 years for determining the requirement of animals. However, the portion of feed companies and research university laboratory applying net energy (NE) system have been increased during the last 10 years because there were many reports reporting the positive effects of NE system as well as decreasing over-evaluation of dietary energy levels in protein and fiber supplements and under-evaluation of dietary energy levels in fat sources (NRC, 2012). Besides, saved energy cost per gain by exact prediction of rate of growth and body composition, decreased pollution source like nitrogen, and decreased protein content in feeds was reported as positive effects of NE system.

Several experiments have been conducted about the application of NE prediction equations for diets and feed ingredients in pigs, and NRC (2012) recommended 2,518 kcal/kg of NE for gestating sows regardless of parity, body weight, backfat thickness and litter size of sows. Then, this requirement was same in all days of gestation and the effective ME and DE requirements for gestating sows were 3,300 and 3,388 kcal/kg of diet, respectively in NRC (2012). In the NE system, the prediction equations are determined from completed diets, and the experiments to apply predictions in individual ingredients are essential. However, there were few studies to determine NE requirement for individual ingredients, and measured heat production can be varied because of limited experimental environment and challenges quantifying fasting heat production (Birkett and de Lange, 2001). Therefore, additional experiments are needed to verify the prediction equations of NE system of many references.

2.2 Changes of Energy Requirements by Thermal Environment

Total heat production is depending on maintenance, heat increment, activity, and maintaining body temperature and dietary requirement of net energy is changeable as heat production increased or decreased (NRC, 2012). A number of factors affect heat production, such as increased energy and protein intake, gastrointestinal tract and liver mass, and there are many studies to evaluate energy expenditure in thermo-neutral environment (Geuyen et al., 1984; Verhagen et al., 1986; Noblet et al., 1997; Peltoniemi et al., 1999; Tummaruk et al., 2004). Lower critical temperature (LCT) and upper critical temperature (UCT) were defined as the minimum and maximum body temperature that could be surmountable by the animals, respectively. Below or above these temperatures, the biological process of cell structures, membranes and enzymes could not maintain usual functions, and these could be a reason of death in animals. Thereby, additional heat production is essential for the sustenance of life.

Noblet et al. (1990) demonstrated that the LCT of individually housed sows were varied from 18 to 23°C as the body weight of sows changed from 150 to 220 kg, and the residential environment including group-housing and strawbedding also had effects on LCT (Geuyen et al., 1984; Verhagen et al., 1986). In case of gestating sows, when sows are in temperatures below the LCT, additional energy is needed for thermogenesis due to restriction feeding method, and the additional energy required for maintaining body temperature ranges from 2.5 to 4.3 kcal of ME/kg$^{0.75}$ per Celsius degree (Noblet et al., 1997). Dourmad et al. (2008) reported that lower critical temperatures (LCT) of gestating sows were 20°C for individually housed sows and 16°C for group-housed sows, respectively, and then the additional maintenance ME requirements were 4.30 and 2.39 kcal/day per 1°C below LCT for individually and group-housed sows, respectively.

The negative effects of high temperature on reproductive performance were suggested many times (Peltoniemi et al., 1999; Tummaruk et al., 2004; Suriyasomboon et al. 2006; NRC, 2012). Suriyasomboon et al. (2006) demonstrated that decreased litter size was observed when sows were raised in high temperature, and Tummaruk et al. (2004) and Peltoniemi et al. (1999) also reported the negative effects of high temperature on reproductive parameters of sows as well as total number of piglets born alive and weaning pigs. The reason of decreased reproductive performance was reduced follicular growth and number of ovulation by heat stress. However, the response of sows about high temperature could be changed easily by the humidity and ventilation rates, and there were few results to evaluating the interaction effects of temperature, humidity and ventilation rates. Therefore, further findings to evaluate the effects of these interactions were needed to determine exact LCT and UCT for gestating sows.

2.3 Dietary Energy Levels in Gestating Sows

Jindal et al. (1996) demonstrated that the gilts fed diets containing constant dietary energy showed decreased embryonic survival rate during early day of gestation with disagreements of Liao and Veum (1994) and Pharazyn et al. (1991)

who presented no detectable effects of dietary energy levels on the number of embryo and the rate of embryo survival. In addition, Dyck and Strain (1983) reported that the gilts fed diets containing moderate energy levels compared to those fed diets containing low energy levels showed increased embryonic mortality and reduced number of embryo from mating to d 10 of gestation, and several studies suggested that decreased rate of embryo survival in gilts fed moderate energy intake could be due to decreased ovulation rate (Toplis et al., 1983). In case of sows, increasing dietary energy levels in early day of gestation had negative effects on the number of embryo survival (Kirkwood et al., 1990) and had no effects on farrowing rate (Dyck and Cole, 1986), However, very low energy intake during early day of gestation had negative effects on litter size (Sorensen and Thorup, 2003) and pregnancy rate (Virolainen et al., 2004). Kongsted (2005) indicated that moderate energy levels of diets during early day of gestation had influences on embryo survival negatively in relatively low prolific sows, whereas high energy diets (above 37 MJ ME day^{-1}) in early pregnancy did not affect the litter size in highly prolific gilts or sows.

Merk and Kirchgessner (1984) investigated that dietary energy levels of 16, 27, or 37 MJ ME day^{-1} from d 43 to 114 of gestation had no detectable effects on litter size with an agreement of Einarsson and Rojkittikhun (1993), and Gatal et al. (1987) found a slightly higher culling rate of gestating sows when they fed high level of energy during mid and late pregnancy because anoestrus after weaning and leg problems were caused by this strategy. Kongsted et al. (2005) suggested that feeding high energy diets during the first 4 wks of gestation have little influences on reproductive performance, and low energy diets for up to fiber parities could not reduce litter size. However, there were also observations showing the positive effects of high energy diets on the risk of being culled when sows were not pregnant repeatedly (Kongsted et al., 2005).

Increased body weight of sows by high energy intake during gestation has been reported many times (Buitrago et al., 1974; Libal and Wahlstrom, 1977; Dourmad, 1991; Van den Brand et al., 2000). Excess energy intake results in increased deposition rate of maternal tissue, body weight and backfat thickness regardless of parity, litter size and species (Dourmad et al., 1999), and impaired body conditions of gestating sows could be a main reason of reproductive problems such as absence of pregnant and abortions and increased culling rate of sows (Young et al., 1990). In addition, there are many studies reporting the negative effects of increased body weight of gestating sows on litter size (King and Young, 1957; Clawson et al., 1963; Ruiz et al., 1968). Feeding high energy diets during gestation decreased voluntary feed intake of sows during lactation, wherein 1 MJ DE/day increase in gestation energy intake was associated with a decrease of 50 g/day or 0.64 MJ/day of feed intake (Baker et al., 1968; Dourmad, 1991). Then, deficient nutrients derived from low feed consumption in lactation can induce reduced amounts of maternal tissue and increased losses of body weight and backfat thickness (Williams, 1995), resulting in reduction of ovulation rate, conception rate and embryo survival in next parity (Hughes et al., 1984; Zak et al., 1997; Han et al., 2000). Generally, the one of purpose for increasing dietary energy level of gestation diet is improving the quality of colostrum and milk. However, Willams (1995) indicated that the chemical composition of milk and colostrum were not affected by dietary energy levels in gestation because sows mobilized their body reserves to support the deficient nutrients.

3. Dietary Methionine Levels and Sources in Lactating Sows

3.1 Importance of Methionine Levels in Lactating Diets

Methionine is second limiting amino acid in swine diet and is classified as sulfur amino acids with cystine. Methionine can meet the total need for sulfur amino acids, because cystine can be synthesized from methionine (but not vice versa). The role of methionine in swine is to support protein synthesis and to be used as a methyl donor group for normal cellular metabolism and the precursor of cystine or carnitine (NRC, 2012). The experiments for evaluating the effects of dietary level of amino acids have been concentrated on lysine (Lewis and Speer, 1975; Wilknison et al., 1992), and there were few studies to evaluate the effects of other amino acids such as threonine, methionine and tryptophan in modern lean genotype sows (Grandhi, 2002). Knabe et al. (1996) demonstrated that dietary lysine levels did not maximize the lactation performance of sows by itself, because the lack of other amino acids such as threonine, methionine and tryptophan also had influences on physiological responses and reproductive performance of sows. Then, Grandhi (2002) presented that ideal ratio of dietary methionine:lysine was lower than 0.27 to maximize lactation performance of modern lean genotype sows, and Schneider et al. (1992) indicated that the sows fed diets containing high level of methionine showed decreased lactation weight loss relative to those fed diets containing low level of methionine, resulting in significant difference (0.16-0.58%). Methionine performs many essential functions especially during gestation and lactation, including methylation of DNA for development, and this process plays a role in the regulation of genetic expression (Ball et al., 2008). Therefore, exact estimation of dietary methionine requirement for gestating and lactating sows is essential in accurately formulating diets for modern sows.

3.2 Requirements of Methionine for Lactating Sows

The method using indicator amino acid oxidation (IAAO) has been used many times to determine the requirements of limiting amino acids for non-ruminant animal (Elango, 2008). Basically, the IAAO technique is based on the concept that when one indispensable amino acid is deficient for protein synthesis, then all other amino acids will be oxidized. The amino acids cannot be stored and must be partitioned between incorporation into protein or oxidation and it means oxidation of the indicator amino acids such as phenylalanine will be decreased with increasing intake of the limiting amino acid. The previous concept about the adult animals, related to the methionine isomers, was completed by the results of IAAO technique, but there are many differences between the current and the past in the many aspects containing genetic traits of sows.

The NRC (1998) requirement for gestating and lactating sows was determined by an assumed ideal protein ratio for sows, because there were no direct estimates of methionine requirement in sows (Ball et al., 2008). Then, the revised requirement of NRC (2012) also used the lactation model, including an assumption for estimated value, and many references and equations were remained to calculate sulfur amino acid requirement without particular change. In these models, to acquire methionine requirement, body weight postpartum, lactation length, average number of nursing piglets, daily piglet weight gain, and voluntary feed intake of lactating sows in individual domestic environment are essential, but the number of pigs weaned and daily litter gain in the references to make these model were intensely low compared with those of the commercial farm in the present as well as 8.0, 9.5 piglets and 1,400, 1,891 g/day, respectively. The parameters acquired from previous observations of the research farm in this study were 3,265 kcal of ME/kg of diet, 235 kg of sow body weight after farrowing, 10.5 of the number of weaned piglets, 2,200 g/day litter gain, and 5.5kg/day of voluntary feed intake of sow, and also exceeded the values of the references in NRC. Generally, a statistician considers that using estimation model is useless when the input area escapes standard area, and it means that estimating the methionine requirement of sows by the lactation model of NRC (1998, 2012) is not proper to modern sows. Ball et al. (2008) reported that the methionine requirement for maintenance of sows could be approximately 14 mg/kg$^{0.75}$, resulting in 40% increase relative to the current requirement of NRC (1998). Thereby, amino acid requirements of modern sows should be re-determined to improve physiological responses and reproductive performance of lactating sows.

3.3 Methionine Sources in Lactating Sows

Except for glycine, all amino acids have isomers in either D or L form, and the utilization efficiency of these forms can vary by different sensitivities of enzyme derived from different three-dimensional configurations between D and L form (NRC, 1998). Usually, naturally occurring proteins has L-form of amino acids, so D-form of amino acids has to be converted to L-form by a two-step enzymatic process, such as oxidative deamination and transamination, for acquiring equivalent efficiency in animal body. Generally, the methionine supplements derived from bacterial fermentation are all in the L-form, but those derived from a chemical process are mixture of D- and L-methionine, called DL-methionine (D:L=50:50). Then, there are many reports to show the equivalence of these two forms (Fell et al., 1959; Featherston et al., 1962; Bauriedal, 1963), and the results were varied by the species and the types of amino acids. Grau and Almquist (1943) demonstrated that D-methionine was metabolically equivalent to L-methionine in chicks with an agreement of other studies (Fell et al., 1959; Bauriedel, 1963), and Dilger and Baker (2007) also showed no significant difference in utilization efficiency between D- and L-methionine.

Many of previous studies have showed that DL-form of methionine could replace L-form without any worry of physiological responses and reproductive performance of pigs except for young pigs (Reifsnyder et al., 1984; Chung and Baker, 1992). Kim and Bayley (1983) demonstrated that D-form was utilized only half as effectively as the L-form in preventing the oxidation of another essential amino acid in piglets, average initial body weight of 1.76 kg. Lunchick et al. (1978) reported that DL-methionine seems to be equivalent to only 80% of L-methionine in young pigs, and Chung and Baker (1992) showed that DL-methionine was utilized with 100% molar efficiency relative to L-methionine without any worry of growth performance in piglets, average initial body weight of 9.6 kg. In the previous findings about the effects of methionine sources on response of young pigs, decreased efficiency of D-form was explained by the moderate enzyme activity and sensitivity related to post-weaning stress and undeveloped digestive system. Then, it means that different utilization efficiency between D- and L-methionine could be found when the enzyme activity and sensitivity are reaching limitation even though the pigs grow up. The maintenance methionine requirement of sows has been increased compared with the past, and Ronald et al. (2008) presented that the maintenance methionine requirement for modern sows was 1.4 times more than requirement of NRC. However, increased activity and sensitivity of enzyme to convert D-methionine to L-methionine have never been reported up to this time. Therefore, further studies to evaluate the changed utilization efficiency of DL-methionine relative to L-methionine by different methionine requirement are needed to make a standard for using methionine supplements in swine feed.

4. Dietary Lysine Levels in Weaning Pigs

4.1 Lysine Levels and Energy Intake

Many experiments were conducted to determine lysine requirements for weaning pigs, and the consequences were varied from 0.87% to 1.27% in pigs weighing 5 to 20 kg (0.87%, 1.15%, 1.11 to 1.27%, 1.10%, 0.95% and 1.27%, respectively; Rogerson and Campbell, 1982; Aherne and Nielsen, 1983; Lin and Jensen, 1985; Thaler et al., 1986; Weaver et al., 1988; Martinez and Knabe, 1990). Voluntary feed intake of pigs is affected by dietary energy levels, and different feed intake induces deficient and excess nutrient intakes even though the requirements of these nutrients are properly controlled (NRC, 1998). Thereby, dietary amino acid levels have to be changed as dietary energy level changed, and then findings related dietary lysine requirement were demonstrated mainly in the aspects of lysine:calorie ratio.

Urynek and Buraczewska (2003) reported that ideal lysine:calorie ratio of pigs weighing 13 to 30 kg was 3.55 g of Lys/Mcal DE, and Nam and Aherne (1994) demonstrated that 3.97 g of Lys/Mcal DE was appropriate for pigs weighing 9 to 26 kg. However, Smith et al. (1999) and Schneider et al. (2010) suggested lysine:calorie ratio of at least 4.35 g for pigs weighing 10 to 25 kg and 4.29 g of Lys/Mcal ME for pigs weighing 9 to 20 kg to achieve the greatest ADG and G/F ratio. Many factors may be associated with this wide variation, but a major reason for this may be the interaction response of dietary energy level and lysine intake (Campbell and Dunkin, 1983; Smith et al., 1999). Dietary amino acid concentrations have influence on feed intake regardless of dietary energy levels, and the results of previous findings might be inconsistent whenever the energy values of feed ingredients were varied. Smith et al. (1999) also agreed with this explanation, and indicated that optimal lysine:calorie ratio could be changed depending on daily caloric intake of the pigs, resulting in ideal lysine:calorie ratio of 4.50 for pigs fed low ME and 4.29 g of Lys/Mcal of ME for pigs fed high ME, respectively.

Once in a while, lysine:calorie ratio was used to control intramuscular fat content, wherein meat quality of the pigs was affected by this trait. In recent years, increasing intramuscular fat content is associated with improving consumer acceptability to pork, especially in South Korea and Japan (Schwab et al., 2006). Numerous studies have reported increased intramuscular fat content as dietary lysine levels and lysine:calorie ratio were decreased in the diets of growing and finishing pigs (Castell et al., 1994; Cineros et al., 1996; Apple et al., 2004; Zhang et al., 2008). However, the negative effects of protein and lysine deficient diets were also reported as well as low feed efficiency and nutrient digestibility, high carcass fat levels and growth check, resulting in decreased productivity in most situations (Jin et al., 2010). Thus, a considerable amount of experiments was needed to find optimal dietary lysine levels and lysine:calorie ratio to increase intramuscular fat content and maintain growth performance and nutrient digestibility.

Consequently, amino acid intake was changed by different dietary energy levels, and many findings were conducted to find optimal lysine:calorie ratio. However, further studies to decrease interaction response of dietary energy level and lysine intake were essential especially for analyzing the effects of these dietary treatments on pigs, because these interaction effects were more than dietary treatment effects.

4.2 Effects of Dietary Lysine Levels on Weaner Diets

There were many publications in evaluating the effects of dietary lysine levels on growth performance, feed efficiency and feed consumption. O'Grady (1978) demonstrated that increasing the lysine level of the diet from 1.10 to 1.18% was associated with improving feed efficiency and weight gain with an agreement of Campbell (1977) who reported optimum pig performance in pigs fed high lysine diets. In addition, Aherne and Nielsen (1983) suggested that lysine requirement for 7 to 19 kg piglets was approximately 1.15% lysine when dietary protein level was 20%, resulting in significant improvement in growth rate and feed efficiency, and Smith et al. (1999) indicated that linear response of ADG and G/F ratio was observed as dietary lysine levels elevated. Although many findings had concentrated on increased ADG and feed efficiency by high lysine diets, there were few researches reporting detectable effects on voluntary feed intake of weaned piglets. By this time, the researches about the relationship between dietary nutrient levels and feed intake of animals had focused on evaluating the effects of dietary energy density (NRC, 1998; Smith et al., 1999; Schneider et al., 2010) and the pigs fed high energy diets generally showed low feed intake relative to those fed low energy diets. However, several findings to investigate the effects of dietary lysine levels had no detectable effects on ADFI in weaned pigs (Aherne and Nielsen, 1983; Schneider et al., 2010).

Nutrient digestibility of animals depends on the types of feed ingredients, dietary nutrient levels, harvesting capacity of gut, age of animals, living environment, and so on, and it is well established that the quality of a protein is primarily associated with its amino acid composition (NRC, 2012). The concept of ideal protein that contains a perfect balance of amino acids, both essential amino acids and non-essential amino acids, had been discussed by numerous researchers (Fuller and Wang, 1990; Cole and Van Lunen, 1994; Lewis, 1995; Baker, 1997).

Table 1. Composition of ideal protein for maintenance, protein accretion and body tissue (NRC, 1998)

Amino acid*	Maintenance	Protein accretion	Body tissue
Arginine**	-200	48	105
Histidine	32	32	45
Isoleucine	75	54	50
Leucine	70	102	109
Lysine	100	100	100
Methionie + cystine	123	55	45
Phenylalanine + tyrosine	121	93	103
Threonine	151	60	58
Tryptophan	26	18	10
Valine	67	68	69

*Proportions of each amino acid relative to lysine were revealed.

**The negative value for arginine reflects arginine synthesis in excess of the needs for maintenance.

In case of pigs fed diets containing cereal grains mainly such as barley, corn, oats, sorghum, or wheat, lysine deficiency could be occurred easily relative to other feed ingredients, wherein first limiting amino acid is lysine in these feed ingredients. Then, nitrogen retention and amino acid availability might be decreased by the broken amino acid balance. The significant effects of dietary lysine levels on digestibilities of dry matter, crude protein, crude ash, and crude fat were not detected in previous findings (Urynek and Buraczewska, 2003), but changed nitrogen retention and blood urea nitrogen were reported many times. Eggum (1970) demonstrated that at least three factors, such as quality and content of protein in the diet, and the time after feeding, have influences on urea content, and Urynek and Buraczewska (2003) observed lower BUN concentration and higher nitrogen retention of pigs fed high lysine diets than those fed low lysine diets. In addition, Jin et al. (1998) found that nitrogen digestibility increased as dietary lysine levels increased up to 1.35% and remained constant beyond 1.35%, and Hansen and Lewis (1993) also reported that linear increase of nitrogen digestibility was observed as dietary CP level increased during growing and finishing phase.

Even though increasing lysine content had positive effects on utilization rate of amino acids, excessive level of this nutrient could increase the amounts of pollution sources as well as excreted nitrogen. Jin et al. (1998) suggested that the amounts of excreted dry matter and nitrogen were significantly affected by the increase of dietary lysine level up to 1.35% ($P<0.05$), where in phosphorus excretion was not affected, and Paik et al. (1996) also demonstrated that nitrogen excretion could be decreased by 10 to 15% through formulating feed closer to requirements. In case of pigs, microbial fermentation usually occurs in the caudal ileum and large intestine (Decuypere and Van der Heyde, 1972). Therefore, the increased amount of excreted nitrogen means decreased amount of digested nitrogen, resulting in increased undigested nitrogen, and then undigested nutrients may be a reason of the production of harmful microbial metabolites. Hobbs et al. (1996) suggested that dietary levels of crude protein and amino acids have effects on the formation and concentration of end products derived from microbial fermentation, and Ball and Aherne (1987) also reported that diets containing high crude protein resulted in undigested protein and encouraged production of pathogenic bacteria in the gut of weaned pigs. Usually, high dietary crude protein levels could be a reason of increased gastric pH due to a high buffering capacity, and this means that harmful bacterial fermentation also could be induced by this dietary treatment (Partanen and Mroz, 1999; Htoo et al., 2007). In general, it is well established that excess dietary nutrient level induces the post-weaning diarrhea of piglets (Lucas and Lodge, 1961; Lecce et al., 1983; Hampson and Beban, 1985), and increased incidence of diarrhea is associated with increased production of amines and ammonia derived from pathogenic fermentation (Porter and Kentworthy, 1969; Dong et al., 1996). In addition, Gaskin (2000) also indicated that increased bacterial fermentation of undigested protein resulted in the production of VFA and toxic substances, such as amines and ammonia, and then they reduced growth performance of weaned pigs. These findings could offer explanations for the effects of excess level of dietary protein and amino acids on incidence of diarrhea after weaning.

Revised lysine requirements of NRC (2012) were 1.53% for 7 to 11 kg piglets fed diet containing 3,400 kcal effective ME/kg and 1.40% for 11 to 25 kg piglets fed that containing 3,350 kcal effective ME/kg. Then, Owen et al. (1995) demonstrated that 1.65% of total dietary lysine resulted in maximum growth rate of weaned pigs weighing 4.6 to 10.1 kg. However, the responses of weaning pigs about dietary lysine requirement were inconsistent immediately after weaning. The weaned piglets from 0 to 2nd wk after weaning could not use dietary nutrients efficiently regardless of the types, because they were suffering from severe post-weaning stress and having an attack of indigestion with the outbreak of diarrhea (Tokach et al., 1995; Beaulieu et al., 2006). After weaning, the effects of dietary treatments are hard to analyze, because the responses of random effects derived from post-weaning stress are larger than those of fixed effects. Therefore, further study will be needed to decrease the effects of post-weaning stress and to evaluate optimal dietary level of lysine in the diets of weaning pigs.

Consequently, feeding high level of dietary lysine had effects on weight gain and feed efficiency, and there was no significant difference in voluntary feed intake in weaned pigs. The piglets fed high lysine diets showed higher nitrogen digestibility and lower BUN than those fed low lysine diets, and excess dietary level of lysine resulted in increased undigested nitrogen and production of harmful microbial metabolites with outbreak of diarrhea and environmental pollution. The responses of dietary treatments were varied when piglets were weaned immediately, because the interaction effects of post-weaning stress were hard to control.

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Chapter III: Effects of Various Energy Levels of Gestating Diet in Different Season on Reproductive Performance and Milk Composition of Sows

ABSTRACT: This experiment was conducted to evaluate the effect of various dietary energy levels of gestating diet in different season on reproductive performance and milk composition of sows. A total of 36 mixed-parity (average = 4.92, range = 3 to 8) sows (F1, Yorkshire × Landrace; Darby, Korea) with an initial body weight (BW) of 218.79 ± 4.74 kg were used for 2 x 4 factorial arrangement of treatments in a split-plot design. The season of mating (Summer-July or Winter-January) was regarded as a main plot and the four different dietary energy levels for gestating sows (3,165, 3,265, 3,365 or 3,465 kcal of ME/kg) were subplots. The experimental diets (Cargill Inc., Korea) containing different energy levels were provided at 2.4kg daily during gestation but lactating diet was provided *ad libitum* regardless of treatments. The lower weight gain from day 0 to 110 of gestation was shown in sows fed low energy diet (linear response, $P<0.05$) and sows mated during winter (season response, $P<0.01$). Both backfat thickness on day 110 of gestation (linear response, $P<0.05$) and backfat gain from day 0 to 110 of gestation (linear response, $P<0.01$) were subsequently shown to be higher when dietary energy level was increased. The changes of backfat thickness during lactation were higher when dietary energy level was increased (linear response, $P<0.01$). However, the litter weight, piglet weight and piglet weight gain during the whole lactating period were not affected by dietary treatments. In the results of the composition of sow colostrum and milk, feeding diets containing different energy levels and mating sows in different seasons had no detectable effects on contents of fat, lactose, protein and total solid except the ratio of solid-not-fat at day 7 postpartum (season response, $P<0.05$) and the fatty acid profiles. The contents of myristic,

stearic and oleic acid in milk were higher when sows were mated during summer and palmitoleic acid at day 14 postpartum was lower as dietary energy level was increased (linear response, $P<0.05$). There was a significant dietary energy level x season interaction in the content of stearic acid at day 7, resulting in high values when dietary energy level was decreased in sows mated during winter compared with those mated during summer. In conclusion, high energy diet feeding during gestation had no effects on reproductive performance in gestating sows regardless of season when environmental temperature in gestating house can be controlled during winter.

Key words: Lactating Sow; Gestating Sow; Mating Season; Energy Level

INTRODUCTION

It is generally well known that reproductive performance of sows depends on several factors, such as genetic traits, parity, temperature and nutrition. In these factors, the temperature of both summer and winter is hard to control in Korea because there are great temperature differences between these two seasons, often exceeding 50°C. There are several studies about the effects of temperature on reproductive performance of sows (Mount, 1972; Sainsbury, 1972). Suriyasomboon (2006) reported that high temperature and humidity at previous weaning, mating or at farrowing had negative effects on litter size. Holmes and McLean (1974) also found that ambient temperature below the low critical would cause increase of heat production, resulting in shortage on energy intake. A considerable amount of researches had been carried out to evaluate the energy requirements of gestating and lactating sows (NRC, 1998) and several standards are established to determine dietary energy levels. When sows are exposed at temperatures below the low critical during winter, additional metabolizable energy will be required compared with those at appropriate temperature. Sainsbury (1972) demonstrated approximately 1,950 kcal of ME/day is needed more to maintain normal condition of sows when the temperature is below 10°C. The consequences of additional energy intake to overcome low environmental temperature during winter on sows have not been well investigated. Moreover, it is not known either there is a relationship between the temperature of the house for sow and energy intake during pregnancy. Therefore, the present study was conducted to determine the effects of various dietary energy levels of gestating diets in different season on reproductive performance and milk composition of sows.

MATERIALS AND METHODS

Animal Management and Experimental Diets

A total of 36 mixed-parity (average = 4.92, range = 3 to 8) sows (F1, Yorkshire × Landrace; Darby, Korea) with an initial BW of 218.79 ± 4.74 kg were used for a trial, at a research farm located in Eum-seong, Korea. The experiment was designed as a 2 x 4 factorial arrangement of treatments in a split-plot design. The season of mating (Summer-July or Winter-January) served as main plot and dietary energy level for gestating sows (3,165, 3,265, 3,365 or 3,465 kcal of ME/kg) was subplot. Sows were allotted to each treatment based on BW, backfat thickness and parity after mating and were housed in an individual gestation stall (2.20 x 0.65 m^2) until day 110 of gestation. On day 110 of gestation, sows were moved into environmentally controlled farrowing rooms and placed in individual farrowing crate (2.50 x 1.80 m^2). Each farrowing crate was equipped with a feeder and a nipple for sows and a heat lamp for newborn piglets. The temperature of the experimental area was presented in Figure 1 and the average daily indoor temperature for gestating sows was 25.5°C in summer and 17.8°C in winter, respectively.

The experimental diets containing different energy levels (3,165, 3,265, 3,365 or 3,465 kcal of ME/kg) controlled by the supplementation of tallow were provided in gestation. For lactation, the same diet was formulated based on corn and soybean meal and contained 3,265kcal of ME/kg, 16.80% crude protein, 1.08% lysine, 0.24% methionine, 0.20% Na, 0.90% Ca, and 0.70% total P, respectively (Table 1). All other nutrients were met or exceeded requirements of NRC (1998). Sows were fed 2.4kg of the experimental diet daily during gestation. However, the same lactation diet was provided *ad libitum* and water was available freely in both periods.

Sample Collections and Analysis

The BW and backfat thickness of sows were measured at day 0 and 110 of gestation and 24 hours postpartum as well as day 21 of lactation. Individual piglet weight and litter size of lactating sows were recorded after postpartum and day 21 of lactation and the weaning to estrus interval (WEI) of sows was checked after weaning as one of important parameters for reproductive performance. Voluntary feed intake of sows was measured during lactation.

Colostrum and milk of sows at day 7, 14 and 21 postpartum were collected from the first and second teats after 5 IU of the oxytocin injection (Komi oxytocin inj. Komipharm international Co., Ltd., Korea). Collected milk samples were also stored at -20°C until later analysis. Proximate analysis of colostrum and milk was conducted using Milkoscan FT 120 (FOSS Electric).

For the analysis of fatty acid composition, samples were prepared using the method of Forch (1957) and were analyzed by the conventional gas chromatography (GC) techniques. Conventional fatty acid analyses were performed on Agilent 7890A GC equipped with a 7683B automatic liquid automatic sampler and flame ionization detector (FID). The Split liner was used for the Split-Splitless Inlet and the GC column was SP-2560 (100m x 0.25mm x 0.25μm; length, I.D and film, respectively). The amount of sample was 1 μl and the nitrogen and hydrogen gases were used as the carrier and detector, respectively.

Statistical Analysis

All of collected data were carried out by least squares mean comparisons and were evaluated with the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). Individual sow and their litter were used as the experimental unit and were analyzed as 2 x 4 factorial arrangements in a split-plot design and the differences were declared significant at $P < 0.05$ or highly significant at $P < 0.01$

and the determination of tendency for all analysis was $P>0.05$ and $P<0.10$. The main plot was the season of mating and the subplot was dietary energy levels of the gestating diets. The effects of increasing dietary energy level were also analyzed as linear and quadratic components by orthogonal polynomial contrasts.

RESULTS

The effect of different dietary energy levels and season of mating on body weight, backfat thickness and weaning to estrus interval (WEI) was presented in Table 2. The weight gain from day 0 to 110 of gestation was affected by dietary energy levels (linear response, $P<0.05$) and season of mating (season response, $P<0.01$), resulting in lower weight gain when dietary energy level was decreased and sows were mated during winter. Both backfat thickness at day 110 of gestation (linear response, $P<0.05$) and the backfat gains from day 0 to 110 of gestation (dietary energy level response, $P<0.05$; linear response, $P<0.01$) were subsequently shown to be higher when dietary energy level was increased. As dietary energy level of gestating diet was increased, the changes of backfat thickness in lactation were higher in linear manner (dietary energy level response, $P<0.01$; linear response, $P<0.01$) and the numerically higher body weight changes were observed in lactation ($P>0.19$).

Although the litter weight, piglet weight and piglet weight gain were not affected by dietary treatment during lactating period (Table 3), the numerically increased numbers of total born and born alive were observed when sows were mated during winter ($P>0.16$). The numbers of nursery pigs after cross-fostering (season response, $P<0.05$) and weaning pigs (season response, $P<0.01$) were also significantly higher when sows were mated during winter. When sows were fed high energy gestating diets tended to have lower feed intake in lactation compared to low energy treatments ($P<0.06$, Figure 2) and there was a numerical increase of voluntary feed intake in lactation when sows were mated during winter (Figure 3).

In the results of the composition of sow colostrum and milk, there were no significant differences in contents of fat, lactose, protein and total solid except solid-not-fat at day 7 postpartum (Table 4). Mating sows during winter had a positive effect on ratio of solid-not-fat in day 7 postpartum ($P<0.05$), but the response was inconsistent, resulting in no detectable effects on the values of the other periods.

Although the milk composition was not changed by dietary treatments, significant responses were observed in fatty acid composition (Table 5). The content of myristic acid at day 7, 14 and 21 postpartum was higher when sows were mated during summer (day 7, $P<0.05$; day 14, $P<0.01$; day 21, $P<0.05$) and this trend was also maintained in the results of palmitic (day 14, $P<0.01$), palmitoleic (day 14, $P<0.05$), stearic (day 7, $P<0.01$, day 14, $P<0.01$) and oleic acid (day 7, $P<0.05$; day 14, $P<0.01$; day 21, $P<0.05$), resulting in a season response. The significant differences in fatty acid profiles by dietary energy treatments were not shown except the results of palmitoleic acid at day 14 postpartum, and shown to be reduced when dietary energy level was increased (linear response, $P<0.05$). However, the other contents of fatty acids were also numerically decreased with the same manner. The content of stearic acid at day 7 was higher when dietary energy level was decreased in sows mated during winter compared with summer groups, resulting in an interaction response ($P<0.05$), and there was no other dietary energy levels X season interaction responses.

DISCUSSION

Feeding high energy diet during gestation and mating sows in summer had effects on increasing BW and backfat thickness in gestating sows. Previous studies indicated that sows fed high energy diet during gestation showed increased body weight and backfat thickness in gestation (Buitrago et al., 1974; Libal and Wahlstrom, 1977; Dourmad, 1991; Van den Brand et al., 2000). Dourmad et al. (1999) reported that surplus energy might be used for deposition of maternal tissue after using maintenance energy and the fetal growth in gestating sows, resulting in the increment of body weight and backfat thickness. The results of the present study agreed with those of previous findings. When lower critical temperature (LCT) of individually housed sows was 20°C, additional maintenance ME requirement was 4.30 kcal/day per 1°C below LCT (Dourmad et al., 2008). In the present study, average daily indoor temperature was 17.8°C in gestation period. This implied that reduced energy for deposition of body tissue by increased energy for maintenance might be caused a decreased backfat thickness and body weight of gestating sows when sows were mated during winter.

In the present study, when sows were fed high energy diets during gestation and mated during summer, decreased voluntary feed intake and higher decreases of body weight and backfat thickness in lactation were observed. Baker et al. (1968) reported that lower feed intake in lactation was observed when dietary energy level in gestation was increased, and Dourmad (1991) demonstrated that the ratio of energy and other nutrients could influence on the appetite of sows and 1 MJ DE/day increase in gestation energy intake was associated with a decrease of 50 g/day or 0.64 MJ/day of feed intake in lactation. The trend of ADFI in this study agreed with those findings. Decreased ADFI during lactation can reduce amounts of maternal tissue, because sows mobilize their body reserves to support the deficient nutrients for maintaining milk contents (Williams, 1995; Lauridsen et al., 2004). In the present study, milk contents of fat, lactose, protein, total solid and solid-not-fat were not differed among treatments despite ADFI was changed by dietary treatment. Then, it might be a reason of increased changes of body weight and backfat thickness in lactation.

Litter size was not affected by dietary energy level, and was increased numerically when sows were mated during winter. Many researches (King and Young, 1957; Clawson et al., 1963; Ruiz et al., 1968) demonstrated that litter size tended to improve when sows were fed high energy diet during gestation, but the response was not consistent. Elsley et al. (1968) and Frobish (1970) represented a significant reduction of the numbers of born alive in the sows fed low energy diets in gestation. Increased litter size had been associated with increased ovulation and fertilization rate (Ulberg and Rampacek, 1974), and previous studies demonstrated that a significant response of dietary energy levels on litter size, supplied high energy diets to sows before mating, causing the changes of circulating insulin concentration and litter size (Cox et al., 1987; Whitley et al., 1998). The lowest daily energy intake of gestating sows (7,596 kcal of ME/day) in the present study was higher than those of previous findings reporting negative effect of low energy intake on litter size after mating. These differences may be important reasons why there is no detectable response on litter size by dietary energy levels in this experiment. Suriyasomboon et al. (2006) demonstrated that high temperature during pregnancy had significantly negative effects on litter size because the heat stress could influence negatively on follicular growth, resulting in decreased number of ovulations. Then, the adverse effect of high temperature on reproduction was also described by several researchers (Peltoniemi et al., 1999; Tummaruk et al., 2004). The results of litter performance in this study agreed with those of previous findings.

The sows, mated during summer, showed high content of long chain fatty acid in milk relative to those mated during winter, and there was no detectable effect of dietary energy level. When sows were mated during summer, farrowing was occurred during winter consequently sows were fed much more lactating diet subsequently high long chain fatty acids in milk were observed. Contents of long chain fatty acid in milk of lactating sows could be changed by diet-derived long chain fatty acids during pregnancy, which are stored in maternal adipose tissue, and lactation (Theil et al., 2004; Amusquivar et al., 2010). Then, Averette et al. (1999) demonstrated that contents of milk fat were not affected by gestating diet when deficient nutrients were replaced by lactating diet. Although sows fed high energy diet or mated during summer showed high BW and backfat thickness, feeding high energy diet, differing from mating sow in summer, had detectable effect on reducing ADFI in lactation. Therefore, the season effect on contents of long chain fatty acid in milk might be due to unchanged feed consumption by mating season.

In this study, there was no dietary energy level x season interaction except the content of stearic acid at day 7 postpartum. Several studies have shown that dietary energy response could be changed by the temperature (Mount, 1972; Sainsbury, 1972). However, the LCT of individually housed sows was changed by housing types, bedding materials, and wall materials (Geuyen et al., 1984; Noblet et al., 1990). In the present study, the gestating sows were caged individually and there was no space between each stall even though gestating house was insulated very efficiently. For these reasons, the indoor temperature was much higher than that of outside temperature in winter (Figure 1) and there were few responses of dietary energy level x season interaction because extra energy was reduced to produce heat.

CONCLUSION

Although dietary energy level of gestating diet was increased from 3,165 to 3,465 kcal of ME/kg of diet, litter size, litter weight and milk composition of sows were not changed except for body weight changes of sows in gestation and lactation. Therefore, dietary energy level of 3,165 kcal of ME/kg is recommended for gestating sows regardless of mating season. The sows inseminated during summer showed increased BW and backfat change in gestation and lactation, elevated contents of long chain fatty acids in milk and decreased litter size, but effects of mating season on these parameters were not improved significantly by dietary energy levels.

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Table 1. The formulas and chemical composition of lactation diet

Ingredients	Lactating diets, g/kg
Corn, yellow	674.2
Soybean meal, 45% CP	246.2
Wheat bran	25.0
Sugar molasses	10.5
Soybean oil	10.0
L-lysine HCl	3.8
Dicalcium phosphate	15.0
Limestone	7.8
Vit. Mix.[1]	2.0
Min. Mix.[2]	1.0
Salt	3.0
Choline chloride, 50%	1.5
Total	1,000.0
Chemical composition[3]	
ME, kcal/kg	3,265.0
CP, g/kg	168.0
Lys, g/kg	10.8
Met, g/kg	2.4
Na, g/kg	2.0
Ca, g/kg	9.0
Total P, g/kg	7.0

[1]Provided per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 1,500 IU; vitamin E, 35 IU; vitamin K, 3 mg; vitamin B_2, 4 mg; vitamin B_6, 3 mg; vitamin B_{12}, 15 µg; pantothenic acid, 10 mg; biotin, 50 µg; niacin, 20 mg; folic acid 500 µg.

[2]Provided per kg of diet: Fe, 75 mg; Mn, 20 mg; Zn, 30 mg; Cu, 55 mg; Se, 100 µg; Co, 250 mg; I, 250 mg.

[3]Calculated values.

Table 2. The effect of energy levels in gestating diets and season of mating on performance and weaning to estrus interval of multiparous sows[1]

Item	Energy level, kcal of ME/kg				SEM[2]	Season		SEM[2]
	3,165	3,265	3,365	3,465		Summer	Winter	
No. Sows	10	9	8	9		19	17	
Body weight, kg								
Gestation								
At mating	219.5	215.8	219.9	220.0	11.08	217.7	220.0	7.63
Day 110 of gestation	265.5	262.2	269.1	276.7	10.06	272.5	264.2	6.93
Gain (d 0-110) [a, b]	46.0	46.4	49.2	56.7	3.84	54.8	44.2	2.65
Lactation								
24 hrs postpartum	246.4	243.3	247.8	257.5	9.66	252.0	245.5	6.66
Day 21 of lactation	242.8	241.9	242.3	247.6	10.07	245.4	241.9	6.94
Changes (d 0-21)	-3.6	-1.4	-5.5	-9.9	3.07	-6.6	-3.6	2.12
Backfat thickness, mm								
Gestation								
At mating	21.1	20.4	20.6	21.0	1.20	20.7	20.8	0.83
Day 110 of gestation [b]	23.4	23.7	25.0	26.9	1.29	25.7	23.8	0.89
Gain (d 0-110) [c, d]	2.3	3.3	4.4	5.9	0.87	5.0	3.0	0.60
Lactation								
24 hrs postpartum	22.3	21.5	23.3	25.1	1.16	24.0	22.0	0.78
Day 21 of lactation	20.8	20.0	20.3	21.1	1.15	20.8	20.2	0.80
Changes (d 0-21) [d, e]	-1.5	-1.5	-3.0	-4.0	0.50	-3.2	-1.8	0.35
WEI, days	5.4	5.2	5.2	5.4	0.24	5.4	5.2	0.17

[1]Initial average weight of sows: 218.79 ± 4.74 kg BW.
[2]Standard error of mean.
[a]Season response ($P < 0.01$).
[b]Dietary energy level linear response ($P < 0.05$).
[c]Dietary energy level response ($P < 0.05$).
[d]Dietary energy level linear response ($P < 0.01$).
[e]Dietary energy level response ($P < 0.01$).

Table 3. The effect of energy levels in gestating diets and season of mating on litter size and growth performance of progeny of multiparous sows

Item	Energy level, kcal of ME/kg				SEM[1]	Season		SEM[1]
	3,165	3,265	3,365	3,465		Summer	Winter	
No. Sows	10	9	8	9		19	17	
Litter size, no. of piglets								
Total born	12.17	12.35	12.75	12.35	0.884	11.70	13.11	0.609
Stillbirth	0.63	0.43	0.38	0.48	0.263	0.30	0.65	0.181
Mummy	-	-	-	-		-	-	
Born alive	11.54	11.93	12.38	11.88	0.755	11.40	12.46	0.520
After cross-fostering [a]	11.29	11.53	11.38	11.50	0.188	11.02	11.83	0.129
Mortality	0.29	0.35	0.13	0.20	0.161	0.37	0.11	0.111
Weaning pigs [b]	11.00	11.18	11.25	11.30	0.223	10.65	11.71	0.154
Litter weight, kg								
At birth	16.49	17.28	17.27	16.59	0.854	16.63	17.19	0.589
After cross-fostering	15.89	16.29	16.02	16.46	0.598	15.95	16.38	0.412
Day 21	61.22	63.59	62.59	60.93	2.355	61.16	63.01	1.623
Weight gain (d 0-21)	45.33	47.30	46.57	44.77	2.411	45.21	46.63	1.661
Piglet weight, kg								
At birth	1.40	1.42	1.40	1.41	0.069	1.48	1.33	0.047
After cross-fostering	1.43	1.45	1.33	1.51	0.139	1.47	1.40	0.095
Day 21	5.58	5.77	5.63	5.50	0.246	5.85	5.39	0.170
Weight gain (d 0-21)	4.17	4.13	4.25	3.85	0.279	4.26	3.94	0.192

[1]Standard error of mean.

[a]Season response ($P < 0.05$).

[b]Season response ($P < 0.01$).

Table 4. The effect of energy levels in gestating diets and season of mating on milk composition during lactation

Item	Energy level, kcal of ME/kg				SEM[1]	Season		SEM[1]
	3,165	3,265	3,365	3,465		Summer	Winter	
Fat, %								
Colostrum	7.33	6.28	7.48	6.91	0.356	6.90	7.10	0.271
Day 7	6.25	6.43	5.76	5.98	0.338	6.46	5.78	0.243
Day 14	5.62	5.97	5.67	5.76	0.257	5.53	6.00	0.192
Day 21	6.23	5.84	5.64	6.06	0.220	5.89	6.03	0.161
Lactose, %								
Colostrum	4.44	4.40	4.16	4.30	0.098	4.28	4.38	0.063
Day 7	5.78	5.69	5.77	5.56	0.089	5.72	5.73	0.062
Day 14	6.04	5.81	5.40	6.09	0.224	5.75	5.97	0.142
Day 21	5.89	5.71	6.10	5.99	0.127	6.01	5.81	0.081
Protein, %								
Colostrum	6.31	7.16	8.32	7.19	0.574	7.24	7.13	0.343
Day 7	4.77	4.92	4.53	4.66	0.130	4.70	4.74	0.092
Day 14	4.33	4.78	4.21	4.40	0.171	4.33	4.55	0.128
Day 21	4.91	4.90	4.62	4.87	0.133	4.83	4.84	0.084
Solid-not-fat, %								
Colostrum	10.98	11.84	12.66	11.75	0.416	11.76	11.75	0.270
Day 7 [a]	10.64	10.70	10.45	10.61	0.830	10.46	10.78	0.060
Day 14	10.45	10.67	9.77	10.60	0.249	10.17	10.64	0.198
Day 21	10.77	10.72	10.77	10.84	0.079	10.77	10.78	0.056
Total solid, %								
Colostrum	20.12	19.99	22.46	20.56	0.617	20.55	20.90	0.453
Day 7	18.34	18.68	17.51	17.91	0.463	18.58	17.72	0.322
Day 14	17.48	18.18	16.68	17.92	0.514	17.33	17.88	0.391
Day 21	18.82	18.08	17.99	18.67	0.333	18.53	18.28	0.227

[1]Standard error of mean.

[a]Season response ($P < 0.05$).

Table 5. The effect of energy levels in gestating diets and season of mating on fatty acid composition (as % w/w of total fatty acids) of colostrum and milk of sows

Item	Energy level, kcal of ME/kg				SEM[1]	Season		SEM[1]
	3,165	3,265	3,365	3,465		Summer	Winter	
Myristic acid (C14:0), %								
Colostrum	1.12	0.87	1.04	1.08	0.091	0.96	1.11	0.051
Day 7[a]	2.79	3.21	2.96	3.22	0.158	3.22	2.83	0.104
Day 14[b]	3.67	3.40	3.13	3.13	0.284	4.11	2.50	0.197
Day 21[a]	3.54	3.07	2.92	3.16	0.223	3.60	2.74	0.143
Palmitic acid (C16:0), %								
Colostrum	17.73	15.16	17.53	16.97	0.971	16.49	17.26	0.622
Day 7	28.41	30.85	28.10	29.55	1.046	30.47	27.86	0.764
Day 14[b]	30.73	27.44	27.45	25.27	2.483	33.68	21.26	1.526
Day 21	31.09	26.00	27.87	27.24	1.810	30.68	25.30	1.310
Palmitoleic acid (C16:1), %								
Colostrum	4.41	3.89	4.18	4.50	0.384	3.98	4.56	0.248
Day 7	10.74	12.01	12.15	12.20	0.814	12.33	11.07	0.517
Day 14 [a, d]	15.09	11.51	10.96	10.31	1.210	14.62	9.24	0.802
Day 21	13.37	11.97	12.30	11.68	0.918	13.68	10.88	0.635
Stearic acid (C18:0), %								
Colostrum	3.91	3.37	4.35	3.98	0.249	4.10	3.66	0.198
Day 7 [b, c]	3.90	3.96	3.74	3.66	0.193	4.25	3.33	0.140
Day 14[b]	3.37	2.52	3.39	2.76	0.246	3.67	2.27	0.183
Day 21	3.00	2.34	2.61	2.47	0.177	2.91	2.29	0.132
Oleic acid (C18:1), %								
Colostrum	32.54	27.04	31.18	31.93	1.679	30.37	31.09	1.292
Day 7[a]	25.26	28.44	27.90	26.52	2.302	31.28	22.13	1.671
Day 14[b]	27.24	22.75	26.70	25.65	2.415	31.97	18.47	1.699
Day 21[a]	25.35	19.33	22.88	21.87	1.634	26.30	18.09	1.235

[1]Standard error of mean.

[a]Season response (P < 0.05).

[b]Season response (P < 0.01).

[c]Dietary energy levels X season interaction (P < 0.05).

[d]Dietary energy level linear response (P < 0.05).

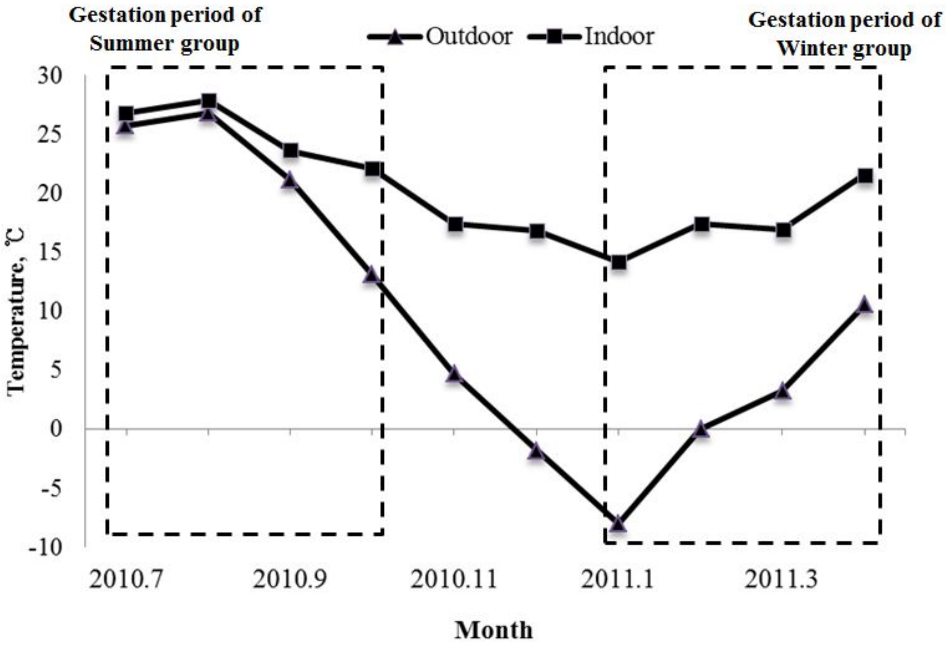

Figure 1. Changes of temperature in experimental area (Eum-seong, Korea)

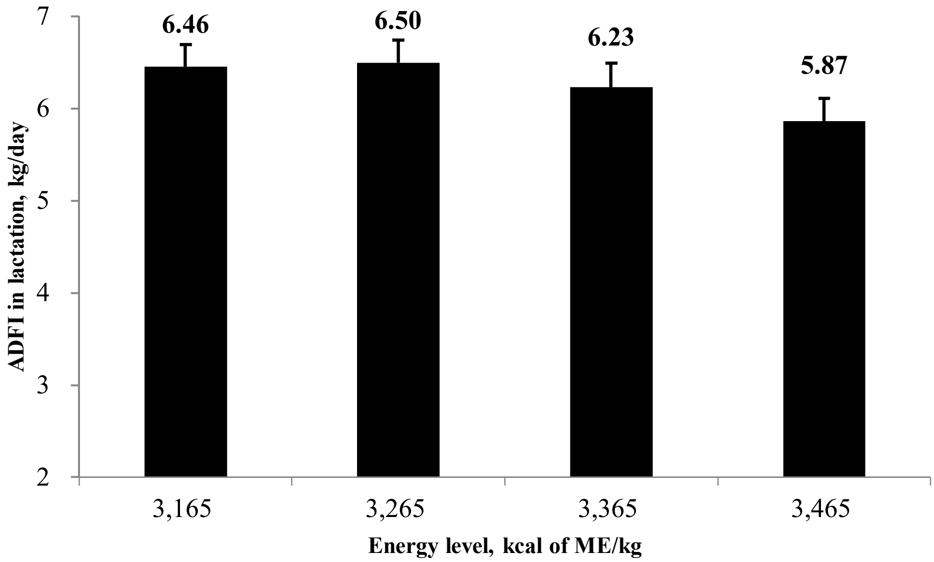

Figure 2. The effect of energy levels in gestating diets on daily feed intake during overall lactating period

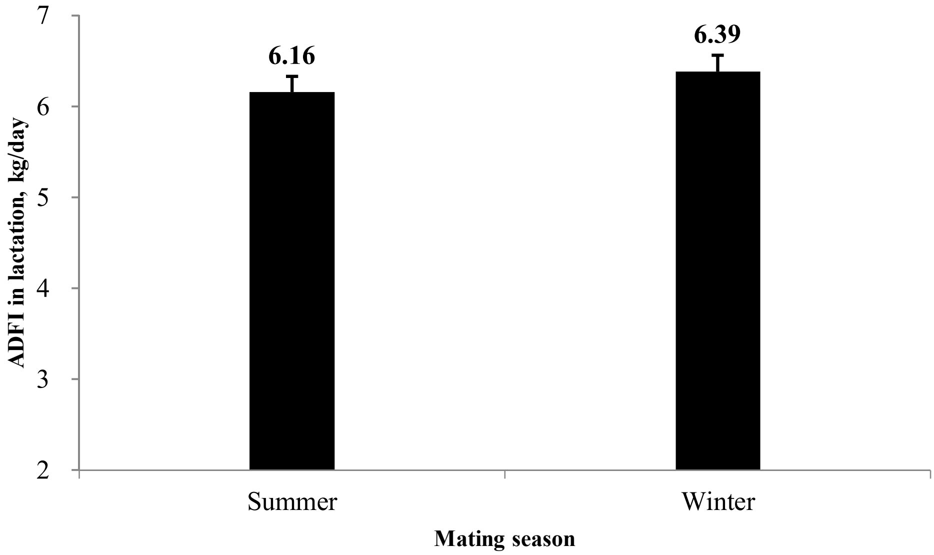

Figure 3. The effect of season of mating on daily feed intake during overall lactating period

Chapter IV: Effects of Dietary Methionine Sources and Supplementation Levels on Body Conditions of Lactating Sows

ABSTRACT: This experiment was done to evaluate the effect of different dietary methionine sources and levels on body conditions of lactating sows. A total of 35 mixed-parity (average = 4.86, range = 2 to 6) gestating sows (F1, Yorkshire × Landrace; Darby, Korea) with an initial BW of 235.17 ± 4.05 kg were used in a 3-wk trial and sows were allotted to one of five treatments based on BW and backfat thickness with 7 replicates by 1 + 2 x 2 factorial arrangement. The experimental treatments were divided by methionine sources and levels; 1) Control: basal diet (NRC requirement), 2) 0.05% DL-methionine: basal diet + 0.05 % DL-methionine, 3) 0.10% DL-methionine: basal diet + 0.10% DL-methionine, 4) 0.05% L-methionine: basal diet + 0.05% L-methionine, 5) 0.10% L-methionine: basal diet + 0.10% L-methionine. The experimental diets were formulated based on corn and soybean meal and contained 3,265 kcal of ME/kg, 16.80% crude protein, 1.08% lysine, 0.20% Na, 0.90% Ca, and 0.70% total P, respectively. All other nutrients were met or exceeded the requirements of NRC (1998). There were no significant differences in body weight and backfat thickness of lactating sows by dietary treatments. The body weight changes in lactation (day 0 to 21) were affected by the supplementation level of L-methionine, resulting in linear and quadratic methionine level responses ($P<0.05$), and then the detectable effect by the supplementation of DL-methionine was also shown, resulting in linear methionine level response ($P<0.05$). Although there were no significant differences, the changes (day 0 to 21 of lactation) of backfat thickness showed similar trend numerically with the body weight changes, and then the weaning to estrus interval (WEI) was shortened when L-methionine was supplemented in lactating diets (sources response, $P<0.05$).

Supplementation of methionine to lactating diet did not show improvements of body weight and weight gain of nursery piglets, and then voluntary feed intake of lactating sows was not affected either by the dietary treatments. The colostrum and milk compositions including milk fat, protein, lactose, total solid and solids-not-fat were not affected by dietary treatments and no differences were found on serum methionine level of piglets in day 21 of lactation. However, when sows were fed diets containing 0.10% DL-methionine, 0.05% and 0.10% L-methionine, higher serum methionine levels were detected compared to those fed control diet ($P<0.01$), resulting in a linear effect by supplementing DL- and L-methionine ($P<0.01$). In methionine level of sow milk, the linear increase was found as dietary L-methionine level increased ($P<0.05$). Consequently, methionine supplementation in lactating diets did not show differences in growth performance of nursery piglets, milk composition and voluntary feed intake of sows regardless of sources and supplementation levels except for body weight changes in gestation, methionine level of serum and milk of lactating sows. The supplementation of L-methionine was more efficient source to reduce body weight loss and to improve the body conditions of lactating sow than that of DL-methionine treatment.

Key words: Lactating Sow; Methionine; Body Conditions; Amino Acid Profiles; Isomer

INTRODUCTION

Modern sows have been selected and improved genetically for high productivity, but the efforts to evaluate changed requirement of methionine for high producing sows were insufficient despite of genetic changes. The NRC (1998) recommended 0.15% dietary methionine level for gilt (average body weight, 150kg; weight gain in gestation, 45kg), but Ronald et al. (2008) suggested that the maintenance methionine requirement for modern sows was 1.4 times more than that of NRC requirement. The methionine requirement of NRC was calculated by ideal protein ratio of assumed equation, not by the accumulated results from many researches. Then, it meant more knowledge about the methionine requirement would be necessary to reflect real situation.

Many studies have been conducted to investigate the effects of crystalline form of methionine, such as L and D-form. D-form has similar bioavailability with L-form (Chung and Baker, 1992), and then Reifsnyder et al. (1984) demonstrated that DL-form could replace the L-form directly to adjust the methionine requirement in swine. However, Bowman et al. (1979) reported that the responses of isomeric effect were not consistent, and Kim and Bayley (1983) indicated that D-methionine had lower bioavailability (50%) relative to L-methionine in reducing phenylalanine catabolism in young pigs. Recent studies about the effect of dietary methionine sources and levels was concentrated on piglet and growing pigs, however, limited information was available for lactating sows.

Therefore, the present study was conducted to evaluate the effect of dietary sources and levels of methionine on productivity of lactating sows and to determine the requirement and ideal form of methionine.

MATERIALS AND METHODS

Animal and Housing

A total of 35 mixed-parity (average = 4.86, range = 2 to 6) gestating sows (F1, Yorkshire × Landrace; Darby, Korea) with an initial BW of 235.17 ± 4.05 kg were used in a 3-wk trial at a research farm located in Eum-seong, Korea. Sows were allotted to one of five treatments based on BW and backfat thickness with 7 replicates in a completely randomized design (CRD). On day 110 of gestation, sows were moved into environmentally controlled farrowing rooms and placed in an individual farrowing crate (2.5 x 1.8 m^2). Each farrowing crate was equipped with a feeder and a nipple waterer for sows and a heat lamp for newborn piglets. Dietary treatments were assigned to the sows after farrowing, and then the BW and backfat thickness of sows were measured at 24 hours postpartum and day 21 of lactation. Individual piglet weight and litter size of sows were recorded at the same time, and then the weaning to estrus interval (WEI) of sows was also measured after weaning as one of important parameters for evaluating reproductive performance.

Experimental Diets and Treatments

For the lactating period, experimental diets were formulated based on corn and soybean meal and contained 3,265 kcal of ME/kg, 16.80% crude protein, 1.08% total lysine, 0.20% Na, 0.90% Ca, and 0.70% total P, respectively. All other nutrients were met or exceeded requirements of NRC (1998). The products of DL-methionine (990 g kg^{-1}) and L-methionine (990 g kg^{-1}) used were from CJ CheilJedang (Seoul, Korea) and was added to the assigned diets at the expense of corn. The formula and chemical composition of experimental diets are presented in Table 1.

The experimental treatments were 1) control: basal diet, 2) 0.05% DL-methionine: basal diet + 0.05% DL-methionine, 3) 0.10% DL-methionine: basal diet + 0.10% DL-methionine, 4) 0.05% L-methionine: basal diet + 0.05% L-methionine, 5) 0.10% L-methionine: basal diet + 0.10% L-methionine.

Sample Collections and Analysis

At day 21 postpartum, blood samples were collected in EDTA tubes from each sow through the jugular vein. Individual sample was centrifuged at 3,000 g on 4°C for 15 min, and then plasma was separated and frozen at -20°C until later analysis. Colostrum and milk of sows were collected from the first and second teats after 5 IU of the oxytocin injection (Komi oxytocin inj. Komipharm international Co., Ltd., Korea). Collected colostrum and milk samples were stored at -20°C until later analysis and proximate analyses of those samples were conducted using Milkoscan FT 120 (FOSS Electric). The methionine level was measured by CJ bio corporation (Korea).

Statistical Analysis

Considering sources and levels of methionine as factors, the data were subjected to factorial analysis. All of collected data were carried out by least squares mean comparisons and were evaluated using PDIFF option in the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). Each individual sow and their litters were used as an experimental unit, and then differences were declared significant at $P<0.05$ and highly significant at $P<0.01$. The effects of increasing levels of methionine were analyzed as linear and quadratic components by orthogonal polynomial contrasts.

RESULTS

The effect of different dietary methionine sources and supplementation levels on body weight, backfat thickness and weaning to estrus interval (WEI) was presented in Table 2. There were no effects of methionine sources and levels on body weight and backfat thickness except for body weight changes of lactating sows, resulting in lower body weight changes in 0.10% DL-methionine, 0.05 and 0.10% L-methionine treatments than that of control treatment ($P<0.05$). The WEI was affected by dietary methionine sources, resulting in lower WEI of the sows fed diets containing L-methionine relative to those fed diets containing DL-methionine ($P<0.05$). When sows were fed diets containing L-methionine, linear and quadratic decreases of body weight changes in lactation were observed ($P<0.05$), and those fed diets containing DL-methionine also revealed a linear decrease of body weight changes in lactation as dietary methionine level was increased ($P<0.05$).

The effect of different dietary methionine sources and levels on piglet performance was shown in Table 3. The piglet performance was not affected by dietary treatments and no detectable differences were observed, but numerically higher litter weight gain was observed in 0.05% and 0.10% DL methionine and 0.05% L-methionine treatments than that of control and 0.10% L-methionine treatments. The voluntary feed intake of lactating sows was not affected by dietary methionine sources and levels, resulting in no significant differences (Figure 1).

The colostrum and milk composition including milk fat, protein, lactose, total solid, and solids-not-fat were not significantly differed as dietary treatments were changed (Table 4), and the numerically higher content of fat, protein, total solid and solids-not-fat were detected in colostrum compared to milk at day 7, 14 and 21 postpartum regardless of treatments.

The effect of different dietary methionine sources and levels on concentrations of plasma of piglet and plasma and milk of lactating sows was shown in Table 5. There was no significant difference in methionine contents both in milk of sows and plasma of piglets in day 21 of lactation, however, the linear increase of milk methionine level was observed by increasing dietary levels of L-methionine as orthogonal polynomial contrast (P<0.05). The higher plasma methionine levels were detected in sows fed diets containing 0.10% DL-methionine, 0.05% or 0.10% L-methionine relative to control treatment (P<0.01) with a main effect of supplementation levels (P<0.01).

DISCUSSION

Dietary methionine level of control treatment in this study was estimated by the lactation model of NRC (1998), and was revised in 2012 NRC. The lactation model needs some information to draw a conclusion such as body weight postpartum, lactation length, average number of nursing piglets, daily piglet weight gain and voluntary feed intake of lactating sows, and the parameters acquired from previous observations of the research farm were used (3,265 kcal of ME/kg; sow body weight after farrowing, 235 kg; the number of weaned piglets, 10.5; litter gain 2,200 g/day, voluntary feed intake of sow, 5.5 kg/day).

In the current study, the sows fed diets containing 0.10% DL-methionine, 0.05% and 0.10% L-methionine treatments showed decreased weight losses in lactation, and decreased WEI after weaning was observed when L-methionine was supplemented to the diets of sows. Grandhi (2002) demonstrated that the modern lean genotype sows could produce higher amounts of milk than conventional sows, subsequently higher level of dietary energy and amino acids were needed. King (1987) and Yang et al. (1989) indicated that the sows mobilized fat and protein derived from body reserves to produce milk when the dietary nutrients were insufficient, resulting in declined rate of conception and embryo survival. Schneider et al. (1992) reported that the decreased weight losses and increased amino acid availability of lactating sows were detected as dietary methionine level was increased from 0.16 to 0.58%. Therefore, reduced weight losses and WEI of sows fed diets containing dietary methionine sources in this study might be associated with different bioavailability of amino acid and nutritional deficiency. In the lactation model of NRC (2012), only two references were selected for empirical estimates of methionine + cysteine requirement, and the number of pigs weaned and daily litter gain of these findings were 8.0, 9.5 piglets and 1,400, 1,891 g/day, respectively. However, this current study estimated methionine requirement based upon 10.5 piglets of the number of pigs weaned and 2,200 g/day, respectively. It seemed that the difference between the calculated requirement and real requirement was increased because of limited references and different inputs.

In the results of the current study, L-form of methionine showed higher efficiency than that of DL-form on body weight losses and plasma methionine levels of lactating sows, resulting in linear and quadratic response of L-form and linear response of DL-form. Previous researches to measure the effects of methionine sources in swine have observed inconsistent results (Bowman, 1979). Kim and Bayley (1983) demonstrated that D-form was utilized only half as effectively as the L-form in preventing the oxidation of another essential amino acid in piglets, average initial body weight of 1.76 kg, and then Lunchick et al. (1978) indicated that DL-methionine was equivalent to only 80% of L-methionine in young pigs. However, Chung and Baker (1992) showed that DL-methionine was utilized with 100% molar efficiency relative to L-methionine without any worry of growth performance in piglets, average initial body weight of 9.6 kg. In previous studies, the reasons related with the efficiency of D-form compared to L-form were not presented, and there were a few evidence about the effect of methionine sources on body conditions and reproductive performance of sows because the researches indicated no difference of utilization efficiency in adult pigs were reported many times (Reifsnyder et al., 1984; Chung and Baker, 1992). In the animal body, D-methionine has to be converted into L-methionine by a two-step enzymatic process, oxidative de-amination and transamination. Then, the bio-efficacy for DL-methionine is determined by this process. In cases of young pigs, the enzyme activity and concentration are not sufficient to convert D-methionine into L-methioinine, and the protein deposition of young animals is higher than that of adult animals, resulting in high protein and amino acid requirements compared to energy levels. Similarly, lactating sows also need high amounts of dietary protein to produce milk protein, and the importance of the second essential amino acids related to use of other amino acids will be increased, especially in modern sows showing greater milk production and litter size (Ronald et al., 2008). These differences of protein accretion and frequency of protein utilization may cause inconsistent results of previous findings and could offer possible explanations for detectable effects of methionine sources on body condition of lactating sows. Further study is needed to demonstrate this effect clearly.

Elliott et al. (1971) reported that the milk composition was not affected by dietary factors. However, linear increase of milk methionine by the supplementation of L-methionine was observed in this experiment. Amino acid utilization in mammary gland is affected by amino acid concentration in blood and mechanisms of amino acid uptake into mammary cells (Hurley et al., 2000), subsequently the amino acid uptake increased as dietary level of amino acid was increased. However, supplementing excess amino acids in lactation diet can reduce amino acid availability in mammary gland (Guan et. al, 2004). Although one linear response of milk methionine was observed in L-methionine, there was no significant difference in results of DL-methionine. The parameters for milk composition such as fat, lactose, protein, solid-not-fat and total solid, showed no significant differences by the dietary treatment. Then, the growth performance of the nursery pigs also showed no significant difference. Based on these results, the dietary methionine sources and levels were not influenced on milk composition of lactating sow and the growth performance of nursery pig.

CONCLUSION

Consequently, methionine supplementation in the diets of lactating sow has no differences in growth performance of nursery piglets, sow milk composition and sow feed intake regardless of sources and levels excepts for sow's body condition and methionine level in plasma and milk. L-methionine is more efficient to reduce body weight loss of lactating sows and to shorten WEI than DL-methionine. This experiment demonstrated that supplementation 0.05% of L-methionine or 0.10% of DL-methionine induced an improvement of body condition for lactating sows.

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Table 1. The formulas and chemical composition of lactation diet[1]

Ingredients, g/kg	Control	DL-methionine 0.05	DL-methionine 0.10	L-methionine 0.05	L-methionine 0.10
Corn, yellow	681.2	681.7	682.1	681.7	682.1
Soybean meal, 45% CP	253.2	252.3	251.6	252.3	251.6
Sugar molasses	10.0	10.0	10.0	10.0	10.0
Soybean oil	11.0	10.9	10.7	10.9	10.7
L-lysine HCl	3.8	3.8	3.8	3.8	3.8
DL-methionine	-	0.5	1.0	-	-
L-methionine	-	-	-	0.5	1.0
Dicalcium phosphate	23.4	23.4	23.4	23.4	23.4
Limestone	8.5	8.5	8.5	8.5	8.5
Vit. Mix.[3]	2.0	2.0	2.0	2.0	2.0
Min. Mix.[4]	1.0	1.0	1.0	1.0	1.0
Salt	4.4	4.4	4.4	4.4	4.4
Choline chloride, 50%	1.5	1.5	1.5	1.5	1.5
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Chemical composition[5]					
ME, kcal/kg	3,265.0	3,265.5	3,265.4	3,265.5	3,265.4
CP, g/kg	168.0	168.0	168.0	168.0	168.0
Lys, g/kg	10.8	10.8	10.8	10.8	10.8
Met, g/kg	2.4	2.9	3.4	2.9	3.4
Met + Cys, g/kg	5.1	5.6	6.1	5.6	6.1
Na, g/kg	2.0	2.0	2.0	2.0	2.0
Ca, g/kg	9.0	9.0	9.0	9.0	9.0
Total P, g/kg	7.0	7.0	7.0	7.0	7.0

[1]Treatments: control (basal diet), 0.05% DL-methionine (basal diet + 0.05% DL-methionine), 0.10% DL-methionine (basal diet + 0.10% DL-methionine), 0.05% L-methionine (basal diet + 0.05% L-methionine), 0.10% L-methionine (basal diet + 0.10% L-methionine).

[2]Lactation feed was provided *ad libitum*.

[3]Provided per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 1,500 IU; vitamin E, 35 IU; vitamin K, 3 mg; vitamin B_2, 4 mg; vitamin B_6, 3 mg; vitamin B_{12}, 15 µg; pantothenic acid, 10 mg; biotin, 50 µg; niacin, 20 mg; folic acid 500 µg.

[4]Provided per kg of diet: Fe, 75 mg; Mn, 20 mg; Zn, 30 mg; Cu, 55 mg; Se, 100 µg; Co, 250 mg; I, 250 mg.

[5]Calculated values.

Table 2. Effects of dietary methionine sources and supplementation levels on body weight, backfat thickness and weaning to estrus interval in lactation

Item[1]		DL-methionine		L-methionine		SEM[2]	P-value[3]		
	Control	0.05	0.10	0.05	0.10		Sou.	Lev.	S x L
No. Sows	7	7	7	7	7				
Body weight, kg									
24 hrs postpartum	233.36	234.50	236.07	237.71	234.21	4.052	0.95	0.92	0.80
Day 21 of lactation	227.93	229.57	234.07	235.86	231.93	4.102	0.84	0.98	0.67
Changes (d 0-21) [*,**,***]	-5.43[c]	-4.93[bc]	-2.00[a]	-1.85[a]	-2.28[ab]	0.454	0.15	0.19	0.09
Backfat thickness, mm									
24 hrs postpartum	20.29	19.57	20.14	21.00	19.71	0.808	0.81	0.86	0.65
Day 21 of lactation	19.14	18.29	19.50	20.71	19.14	0.882	0.64	0.94	0.53
Changes (d 0-21)	-1.15	-1.28	-0.64	-0.29	-0.57	0.173	0.19	0.66	0.25
WEI, days	5.29	5.36	5.00	4.71	4.86	0.105	0.05	0.58	0.20

[a,b,c] means with different superscripts within the same row significantly differ ($P<0.05$).

[1]Treatments: control (basal diet), 0.05% DL-methionine (basal diet + 0.05% DL-methionine), 0.10% DL-methionine (basal diet + 0.10% DL-methionine), 0.05% L-methionine (basal diet + 0.05% L-methionine), 0.10% L-methionine (basal diet + 0.10% L-methionine).

[2] Standard error of mean.

[3]Abbreviation: Sou. (methionine source), Lev. (methionine level), and S X L (interaction between methionine source and level).

[*]Linear effect by the supplementation of DL-methionine ($P<0.05$).

[**]Linear effect by the supplementation of L-methionine ($P<0.05$).

[***]Quadric effect by the supplementation of L-methionine ($P<0.05$).

Table 3. Effects of dietary methionine sources and supplementation levels on piglet performance

Item[1]	Control	DL-methionine		L-methionine		SEM[2]	P-value[3]		
		0.05	0.10	0.05	0.10		Sou.	Lev.	S x L
Litter weight, kg									
After cross-fostering	15.9	16.2	16.2	16.4	16.5	0.392	0.65	0.96	0.87
Day 21 of lactation	61.6	62.9	65.0	63.9	61.8	1.114	0.81	0.87	0.36
Weight gain, kg/d	2.18	2.22	2.32	2.26	2.16	0.041	0.51	0.72	0.35
Piglet weight, kg									
After cross-fostering	1.50	1.53	1.54	1.58	1.56	0.037	0.71	0.94	0.99
Day 21 of lactation	5.80	5.96	6.16	6.14	5.86	0.110	0.65	0.99	0.36
Weight gain, kg/d	0.20	0.21	0.22	0.22	0.20	0.004	0.47	0.99	0.26

[1]Treatments: control (basal diet), 0.05% DL-methionine (basal diet + 0.05% DL-methionine), 0.10% DL-methionine (basal diet + 0.10% DL-methionine), 0.05% L-methionine (basal diet + 0.05% L-methionine), 0.10% L-methionine (basal diet + 0.10% L-methionine).

[2] Standard error of mean.

[3]Abbreviation: Sou. (methionine source), Lev. (methionine level), and S X L (interaction between methionine source and level).

Table 4. Effects of dietary methionine sources and supplementation levels on milk composition in lactation

Item[1]	Control	DL-methionine		L-methionine		SEM[2]	P-value[3]		
		0.05	0.10	0.05	0.10		Sou.	Lev.	S x L
Fat, %									
Colostrum	6.93	7.38	6.71	6.69	7.32	0.232	0.94	0.98	0.27
Day 7	6.59	6.36	6.81	6.22	6.85	0.144	0.87	0.12	0.79
Day 14	6.58	6.54	6.56	6.73	6.41	0.151	0.96	0.67	0.64
Day 21	6.11	5.87	6.08	6.06	6.07	0.112	0.74	0.70	0.71
Lactose, %									
Colostrum	4.25	4.07	4.02	4.48	4.26	0.087	0.10	0.48	0.67
Day 7	5.84	5.74	5.65	5.81	5.65	0.032	0.19	0.17	0.93
Day 14	5.88	5.93	5.89	5.86	5.81	0.034	0.33	0.61	0.93
Day 21	5.87	5.93	5.95	5.93	5.95	0.026	0.98	0.82	1.00
`Protein, %									
Colostrum	6.72	7.32	7.11	6.23	7.57	0.271	0.64	0.40	0.25
Day 7	4.35	4.76	4.55	4.53	4.63	0.064	0.52	0.62	0.19
Day 14	4.67	4.52	4.46	4.38	4.46	0.052	0.57	0.93	0.58
Day 21	4.68	4.68	4.46	4.66	4.67	0.058	0.51	0.44	0.44
Solid-not-fat, %									
Colostrum	10.92	11.63	12.56	10.63	12.04	0.311	0.29	0.11	0.74
Day 7	10.47	10.71	10.47	10.58	10.59	0.056	0.98	0.29	0.25
Day 14	10.77	10.64	10.60	10.47	10.52	0.044	0.19	0.94	0.65
Day 21	10.79	10.79	10.60	10.78	10.80	0.042	0.34	0.38	0.32
Total solid, %									
Colostrum	20.61	21.13	20.48	19.40	21.49	0.376	0.68	0.42	0.13
Day 7	18.28	18.31	18.52	18.11	18.75	0.162	0.97	0.26	0.57
Day 14	18.66	18.44	18.42	18.46	18.11	0.181	0.74	0.68	0.71
Day 21	18.16	18.03	18.02	18.51	18.09	0.152	0.46	0.55	0.58

[1]Treatments: control (basal diet), 0.05% DL-methionine (basal diet + 0.05% DL-methionine), 0.10% DL-methionine (basal diet + 0.10% DL-methionine), 0.05% L-methionine (basal diet + 0.05% L-methionine), 0.10% L-methionine (basal diet + 0.10% L-methionine).

[2] Standard error of mean.

[3]Abbreviation: Sou. (methionine source), Lev. (methionine level), and S X L (interaction between methionine source and level).

Table 5. Effects of dietary methionine sources and supplementation levels on methionine level of plasma of piglet and milk and plasma of lactating sows

Item[1]		DL-methionine		L-methionine		SEM[2]	P-value[3]		
	Control	0.05	0.10	0.05	0.10		Sou.	Lev.	S x L
Sow plasma, mg/L[*,**]									
Day 21 of lactation	4.95[c]	6.79[bc]	9.57[a]	7.73[ab]	9.33[a]	0.425	0.66	0.01	0.46
Sow milk, mg/L									
Day 21 of lactation[***]	2.24	3.18	2.90	2.13	3.47	0.222	0.65	0.31	0.13
Piglet plasma, mg/L									
Day 21 after birth	10.54	11.12	10.77	14.32	10.86	0.638	0.28	0.21	0.30

[a,b,c] means with different superscripts within the same row significantly differ (P<0.01)

[1]Treatments: control (basal diet), 0.05% DL-methionine (basal diet + 0.05% DL-methionine), 0.10% DL-methionine (basal diet + 0.10% DL-methionine), 0.05% L-methionine (basal diet + 0.05% L-methionine), 0.10% L-methionine (basal diet + 0.10% L-methionine).

[2] Standard error of mean.

[3]Abbreviation: Sou. (methionine source), Lev. (methionine level), and S X L (interaction between methionine source and level).

[*]Linear effect by the supplementation of DL-methionine (P<0.01).

[**]Linear effect by the supplementation of L-methionine (P<0.01).

[***] Linear effect by the supplementation of L-methionine (P<0.05).

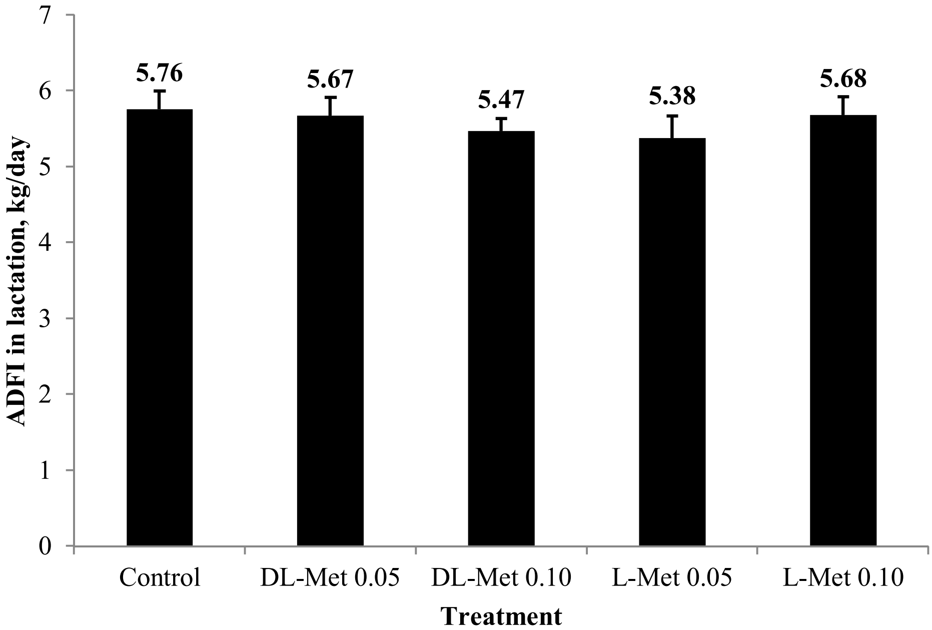

Figure 1. Effects of dietary methionine sources and levels on daily feed intake in lactation

Chapter V: Effects of Dietary Lysine Levels on Growth Performance, Nutrient Digestibility, Blood Profiles, Fecal *E.coli* Counts and Incidence of Diarrhea in Weaning Pigs

ABSTRACT: This study was conducted to invesetigate the effects of dietary lysine levels on physiological responses, nutrient digestibility, blood profiles, fecal *E.coli* counts, and incidence of diarrhea in weaning pigs. A total of 128 piglets [(Yorkshire × Landrace) × Duroc] (BW = 7.24 ± 0.03 kg; weaned at day 28 ± 2) were randomly allocated to one of four treatments in a randomized complete block (RCB) design and 8 replicates with 4 pigs per pen. Dietary treatments were divided by the dietary level of lysine; 1) N + 0.15% (NRC requirement + 0.15% lysine), 2) N (NRC requirement), 3) N – 0.15% (NRC requirement – 0.15% lysine), 4) N – 0.30% (NRC requirement – 0.30% lysine). The Phase I (0-2nd wk after weaning) diet containing 3,265 kcal of ME/kg and 23.70% crude protein and Phase II (3rd–5th wk after weaning) diet containing 3,265 kcal of ME/kg and 20.90% crude protein were provided *ad libitum* during experimental period. All other nutrients were met or exceeded requirements of NRC (1998). There were no significant differences in ADG, ADFI and G/F ratio of Phase I. However, ADG and G/F ratio at Phase II were slightly decreased as dietary lysine level was reduced, resulting in linear ($P<0.05$) and quadratic responses ($P<0.05$). Feeding of high lysine diets had effects on reducing BUN concentration (linear responses at 2nd and 5th wks and quadratic response at 5th wk, $P<0.01$) and urinary nitrogen (linear response, $P<0.05$), whereas the nutrient digestibilities of dry matter, crude protein, crude ash, and crude fat were not affected by dietary treatments. The piglets fed high lysine diets showed higher moisture content in feces at 2nd wk ($P<0.05$), fecal *E.coli* counts at 5th wk ($P<0.01$), and showed higher incidence of diarrhea ($P<0.05$) compared with low lysine diets. Consequently, these results suggested that dietary lysine requirement would be for

approximately 1.15% lysine/kg of diet when 11 to 20 kg of BW pigs fed diets containing 20.9% crude protein and piglets fed high lysine diets showed higher nitrogen retention, but incidence of diarrhea, and higher fecal *E.coli* counts were also increased compared to low lysine treatment diets, resulting in detrimental effects on GI tract of weaning pigs.

Key words: Lysine; Weaning Pigs; Nitrogen Retention; Nutrient Digestibility; Growth Performance

INTRODUCTION

Weaning of the pigs is often associated with growth check, decreased nutrient digestibility, and incidence of diarrhea due to immature gastrointestinal tract, social stress and immunological challenge (Aherne et al., 1992; Htoo et al., 2007). Then, optimal amino acid intake and amino acid compositions to improve the availability of amino acid are very important issues after weaning (NRC, 1998), because bone and tissue of piglets are rapidly developed in this period. Dewey et al. (1993) demonstrated that increasing lysine intake to improve amino acid balance is needed to achieve maximum rate of growth, and Gahl et al. (1992) reported that lysine was essential nutrient to synthesize muscle protein and is easy to accumulate in the body. However, there were several findings reporting the negative effects of lysine surplus, such as growth check, increased feed cost, and nitrogen excretion (Bin and Tian, 2003; Jin, 2010). Then, Hobbs et al. (1996) suggested that the incidence of diarrhea could be increased by changed fermentation product derived from different dietary protein or lysine levels. Therefore, evaluating lysine requirement for weaned piglets is more important than other nutritional strategies to improve physiological responses of them.

It is generally well known that amino acid availability of pigs could differ mainly depending on dietary lysine levels, wherein this nutrient is first limiting amino acids when main ingredients of feed for swine are corn, barley, oats, sorghum and wheat (Lewis, 1985; Southern, 1991; Kerr, 1995). The NRC (1998) recommended 1.15% of lysine as a nutrient requirement for piglets weighing 5 to 20 kg when dietary energy level was 3,265 kcal of ME/kg, and revised lysine requirements of NRC (2012) suggested 1.53% for 7 to 11 kg piglets fed diet containing 3,400 kcal effective ME/kg and 1.40% for 11 to 25 kg piglets fed that containing 3,350 kcal effective ME/kg. Thus, many attempts have been made to determine lysine requirement for piglets, but there were few studies to evaluate dietary lysine requirement in domestic environment and situation, and the results of these findings were inconsistent. Therefore, this experiment was conducted to investigate the effects of dietary lysine levels on physiological responses, nutrient digestibility, blood profiles, fecal *E.coli* counts, and incidence of diarrhea in weaning pigs.

MATERIALS AND METHODS

Experimental Design, Diet, and Management

A total of 128 weaning pigs ([Yorkshire × Landrace] × Duroc; 7.24 ± 0.03 kg initial BW) weaned at day 28 ± 2 were used in a 5-wk growth trial, at a research farm located in Suwon, South Korea. Piglets were blocked by body weight and gender and allotted to one of four treatments in 8 replicates with 4 pigs per pen in a randomized complete block design (RCBD). Dietary treatments were divided by dietary lysine levels as followings; 1) N + 0.15% (NRC requirement + 0.15% lysine), 2) N (NRC requirement), 3) N – 0.15% (NRC requirement – 0.15% lysine), 4) N – 0.30% (NRC requirement – 0.30% lysine). The diets containing 3,265 kcal of ME/kg and 23.70% crude protein and the other diets containing 3,265 kcal of ME/kg and 20.90% crude protein were supplied for the Phase I and II, respectively. All other nutrients were met or exceeded requirements of NRC (1998). The experimental diets and chemical composition are presented in Tables 1 and 2.

All pigs were housed in an environmentally controlled building with half-slotted concrete floors (0.90 x 2.15 m^2) and each pen was equipped with a feeder and a nipple drinker to provide water and feed with *ad libitum* access. Body weight and feed intake were recorded at 0, 2nd and 5th wk to calculate the average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G/F ratio).

Metabolism Trial

For evaluating total tract digestibility, a total of 16 piglets ([Yorkshire × Landrace] × Duroc; 10.21 ± 0.37 kg initial BW) were split into four treatments with completely randomized design (CRD). All pigs were housed in an individual metabolic crate in a room of steady temperature (27 ºC), controlled with a heating lamp. The experimental diets were supplied twice a day at 0700 and 1900 with *ad-libitum* access to water according to the rate of 2.0 times of the maintenance requirement for ME (NRC, 1998) based on initial BW of pigs. After 6 days of adaptation period, piglets were subjected to 5 days collection and chromic oxide and ferric oxide were used as initial and end marker, respectively. Collected excreta were stored at -20℃ during the collection period and dried (60 ºC, 72 h) and ground (2 mm screen, Wiley mill) for chemical analysis at the end of trial.

Blood Urea Nitrogen (BUN)

Blood samples were collected from anterior vena cava of the same pigs at 0, 2nd and 5th wks for BUN analysis. Collected blood samples were quickly centrifuged for 15 min at 3,000 rpm and 4 ºC. The serum was carefully removed to plastic vials and stored at -20 ºC until BUN analysis. Total BUN concentrations were analyzed using blood analyzer (Ciba-Corning model, Express Plus, Ciba Corning Diagnostics Co.).

Moisture Contents of Feces

Fecal samples were collected from 6 piglets per treatment by rectal dilatation during 24 h. 1.0 g of sample was dried in a forced air drying oven at 105 ºC for 3 h and analysis of fecal moisture was carried out by the AOAC (1995) procedure.

* Moisture content (%) = (1 g sample weight – dried 1g sample weight) × 100 / 1 g sample weight

Incidence of Diarrhea

The incidence of diarrhea was checked every day at 0700 and score of diarrhea was given into 5 numbers by counting pigs showing evidence of watery diarrhea (0, 1, 2, 3 and 4; the numbers of pigs suffering diarrhea in each pen).

Fecal E.coli Counts

Fecal *E.coli* counts were analyzed at 0, 2nd and 5th wk, respectively. For enumeration of bacterial populations, 1 g of fecal content was diluted in saline solution and re-suspended by vortex mixing, then serially diluted in saline solution. Each dilution was spread overlaid on the surface of an agar plate and this procedure was performed in duplicate over *Lactobacillus* MRS broth and MacConkey agar (Difco, BD science, USA). Plates were incubated at 37°C and each plate was spotted for fecal micro-flora count and examined for the presence of each bacterial growth after 24 h of incubation.

Chemical and Data Analysis

Experimental diet and excreta were analyzed for contents of dry matter (procedure 967.03; AOAC, 1995), ash (procedure 923.03; AOAC, 1995), N by using the Kjeldahl procedure with Kjeltec (KjeltecTM 2200, Foss Tecator, Sweden) and CP content (N × 6.25; procedure 981.10; AOAC, 1995). The experimental data were carried out by least squares mean comparisons and evaluated using PDIFF option using General Linear Model (GLM) procedure of SAS (2004). In case of growth performance, a pen was considered as an experimental unit, while individual piglet was used as a unit to analyze nutrient digestibility, BUN, moisture content of feces, incidence of diarrhea and fecal *E.coli* counts. The effects of increasing levels of lysine were analyzed as linear and quadratic components by orthogonal polynomial contrasts. Differences were declared significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS

The effects of dietary lysine levels on ADG, ADFI and G/F ratio during experimental period was shown in Table 3. The feed consumption was not affected by increasing levels of dietary lysine. However, average body weight at 5th wk and ADG of Phase II (3rd-5th wk) and during the whole experimental period was reduced in pigs fed diets containing low level of dietary lysine, resulting in linear and quadratic responses ($P<0.05$). The G/F ratio was also declined as dietary level of lysine was decreased with linear and quadratic response at Phase II and overall period ($P<0.05$).

The pigs fed diets containing high level of dietary lysine were showed lower BUN concentration compared to those fed low lysine treatment diets (Table 4; linear responses at 2nd and 5th wks and quadratic response at 5th wk, $P<0.01$). Although the apparent total tract digestibility of dry matter, crude protein, crude ash and crude fat were not influenced by dietary treatments, linear increase of urinary nitrogen and linear decrease of nitrogen retention were detected as dietary lysine level was reduced (Table 5; linear response, $P<0.05$).

Feeding diets containing low level of dietary lysine reduced moisture content of feces at 2nd wk with linear response ($P<0.05$) and there was no detectable effect on those results at 5th wk (Table 6). Moreover, the pigs fed diets containing high level of dietary lysine showed higher diarrhea score at Phase I (0-2nd wk) and fecal *E.coli* counts at 5th wk relative to those fed diets containing low level of dietary lysine, resulting in linear response of dietary lysine level ($P<0.05$).

DISCUSSION

Many experiments have been conducted to determine lysine requirement of piglets and optimal lysine:ME ratio, and the consequences for lysine requirement were ranged from 0.87% to 1.27% in pigs weighing 5 to 20 kg (Rogerson and Campbell, 1982; Aherne and Nielsen, 1983; Lin and Jensen, 1985; Thaler et al., 1986; Weaver et al., 1988; Martinez and Knabe, 1990; Owen et al., 1995). Then, the NRC (1998) recommended 1.15% of lysine as a nutrient requirement for piglets weighing 5 to 20 kg when dietary energy level was 3,265 kcal of ME/kg. However, revised lysine requirements of NRC (2012) were 1.53% for 7 to 11 kg piglets fed diet containing 3,400 kcal effective ME/kg and 1.40% for 11 to 25 kg piglets fed that containing 3,350 kcal effective ME/kg. In the present study, ADG, ADFI and G/F ratio were not changed by dietary treatment in Phase I (0-2nd wk), whereas ADG and G/F ratio were decreased linearly ($P<0.05$) and quadratically ($P<0.05$) as dietary lysine level was lowered in Phase II (3rd-5th wk). Then, these linear responses of Phase II are in agreement with those of Smith et al. (1999) who reported linear increase of feed efficiency for 10 to 25 kg piglets fed diets containing various dietary energy levels ranged from 3,250 to 3,510 kcal of ME/kg and Schneider et al. (2010) who presented linear increases of ADG and G/F ratio for those (average initial body weight = 9.30 kg) fed diet containing 2,950 kcal of ME/kg.

According to Beaulieu et al. (2006) and Tokach et al. (1995), the responses of piglets about dietary nutrient levels were inconsistent because piglets from 0 to 2nd wk after weaning could not use dietary nutrients efficiently and they were suffering social stress, and these findings might be an explanation for unchanged ADG and G/F ratio by dietary treatments of this study in Phase I. Schneider et al. (2010) demonstrated that optimal lysine:ME ratio might differ depending on dietary energy levels of diets, and Aherne and Nielsen (1982) reported that lysine requirement for 7 to 19 kg piglets was approximately 1.15% lysine when dietary protein level was 20%. Similarly, decreased ADG and G/F ratio were observed when the piglets were fed low lysine diets in the present study, and these parameters were the highest in those fed diet containing 1.15% lysine/kg in Phase II, resulting in linear and quadratic responses. The researches about the relationship between dietary nutrient levels and feed intake of animals have focused on determining the effects of dietary energy density (NRC, 1998; Smith et al., 1999; Schneider et al., 2010) and it is generally well known that high energy density in diet reduces daily feed intake of piglets. However, several studies of dietary lysine levels suggested that ADFI of weaned piglets was not affected by these treatments (Aherne and Nielsen, 1982; Schneider et al., 2010), and the significant effects of dietary lysine levels on ADFI was not detected in this study with an agreement of previous findings.

Dietary lysine level effects observed in the results of BUN and N-retention were similar to those demonstrated by Urynek and Buraczewska (2003) and Eggum (1970), wherein high lysine diets resulted in lower BUN concentration and higher N-retention than low lysine diets. The concept of the ideal protein was already presented often in the previous studies (Fuller and Wang, 1990; Cole and Van Lunen, 1994; Baker, 1997), and it is well established that nutritional value and utilization efficiency of amino acids are primarily dependent on amino acids profiles and composition. Especially, lysine is most important amino acid to control the protein quality because it is a first limiting amino acid in swine fed diets containing cereal grains such as barley, corn, oats, sorghum, or wheat, and dietary lysine levels could affect protein availability and the rate of surplus amino acids in urine and blood of piglets. In this study, although linear response of dietary lysine level on serum BUN concentration was detected in Phase I and II, the quadratic response was also checked in Phase II. Then, it meant 1.15% lysine/kg of diet was sufficient to utilize amino acids efficiently in the piglets weighing 11 to 20 kg approximately when they were fed diets containing 20.9% of crude protein, resulting in no difference compared to those fed diets containing 1.30% lysine/kg of diet. Even though there were many findings to evaluate the effects of dietary lysine levels on nitrogen retention and protein quality, limited information is available to assess nutrient digestibility such as dry matter, crude protein, crude ash, or crude fat. Then, there was no detectable effect in the results of these few studies about nutrient digestibility which agreed with the consequences of this study (Urynek and Buraczewska, 2003).

Ball and Aherne (1987) suggested that the incidence of diarrhea could be increased by high level of dietary nutrients and low digestibility of nutrients, and then Hobbs et al. (1996) reported that dietary CP levels might affect microbial fermentation and the metabolite amounts of these microbes. In case of weaning pigs, undigested protein could encourage proliferation of pathogenic bacteria (Ball and Aherne, 1987), and bacterial fermentation of this nutrient could increase the amounts of amines and ammonia which can induce growth check of piglets (Gaskins, 2000). In addition, these toxic substances also could be a reason of high incidence of diarrhea (Porter and Kenworthy, 1969; Dong et al., 1996). In the present study, the digestibility of crude protein was not affected by dietary treatments, but ADFI of piglets showed numerical increase when pigs were fed high lysine diet in Phase II, resulting in increased amounts of undigested protein. These might be a reason of higher counts of fecal *E.coli* in N+0.15% and N treatments relative to those in N-0.15% and N-0.30% treatments, resulting in linear lysine level response in Phase II. In Phase I, the piglets fed high lysine diets showed higher moisture contents and the incidence of diarrhea than those fed low lysine diets, but various dietary level of lysine had no beneficial effects on fecal

E.coli counts because the piglets showed high fecal *E.coli* counts regardless of treatments.

CONCLUSION

Dietary lysine levels had no effects on ADFI and nutrient digestibility in weaning pigs. However, high lysine diets increased ADG, G/F ratio, N retention and fecal *E. coli* counts in Phase II (3rd-5th wk), and increased moisture content and incidence of diarrhea in Phase I (0-2nd wk). Consequently, detectable effect of high dietary lysine level was not found in Phase I, and recommended dietary lysine requirement was approximately 1.15% total lysine/kg of diet when 11 to 20 kg of BW pigs were fed diets containing 20.9% crude protein.

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Table 1. The formulas and chemical composition of experimental diet in Phase I

Ingredients, g/kg	Treatments[1]			
	N + 0.15%	N	N – 0.15%	N – 0.30%
EP corn	162.1	166.4	170.6	173.4
Soybean meal, 44% CP	346.0	332.4	319.2	306.4
Whey powder	20.0	20.0	20.0	20.0
Corn gluten meal	43.8	56.0	67.8	79.6
Lactose	120.0	120.0	120.0	120.0
Barley	274.0	274.0	274.0	274.0
Soybean oil	2.2	1.1	-	-
MCP	10.9	10.9	10.9	10.9
Limestone	9.2	9.2	9.3	9.3
L-Lysine·HCl	5.1	3.4	1.7	-
DL-methionine	0.3	0.2	0.1	-
Vit. Mix[2]	1.2	1.2	1.2	1.2
Min. Mix[3]	1.2	1.2	1.2	1.2
Salt	2.0	2.0	2.0	2.0
Choline chloride, 25%	1.0	1.0	1.0	1.0
ZnO	1.0	1.0	1.0	1.0
Total	1,000.0	1,000.0	1,000.0	1,000.0
Chemical composition[4]				
ME, kcal/kg	3,265.1	3,265.8	3,265.9	3,265.8
CP, g/kg	237.0	237.0	237.0	237.0
Lys, g/kg	15.0	13.5	12.0	10.5
Met, g/kg	3.8	3.8	3.8	3.8
Ca, g/kg	8.0	8.0	8.0	8.0
Total P, g/kg	6.5	6.5	6.5	6.5

[1] Treatments: N + 0.15% (NRC requirement + 0.15% lysine), N (NRC requirement), N – 0.15% (NRC requirement – 0.15% lysine), N – 0.30% (NRC requirement – 0.30% lysine).

[2] Provided per kg of diet: 8,000 IU of vitamin A; 1,600 IU of vitamin D_3; 32 IU of vitamin E; 64 g of D-biotin; 3.2 mg of riboflavin; 8 mg of calcium-pantothenic acid; 16 mg of niacin; 12 g of vitamin B_{12}; 2.4 mg of vitamin K.

[3] Provided per kg of diet: 127.3 mg of Fe; 54.1 mg of Cu; 24.8 mg of Mn; 84.7 mg of Zn; 0.3 mg of Co; 0.3 mg of I; and 0.1 mg of Se.

[4] Calculated values.

Table 2. The formulas and chemical composition of experimental diet in Phase II

Ingredients, g/kg	Treatments[1]			
	N + 0.15%	N	N – 0.15%	N – 0.30%
EP corn	339.2	337.5	335.7	336.6
Soybean meal, 44% CP	223.4	224.5	225.6	219.2
Corn gluten meal	65.7	68.2	70.7	78.0
Lactose	40.0	40.0	40.0	40.0
Barley	300.0	300.0	300.0	300.0
Soybean oil	3.4	3.5	3.7	3.8
MCP	10.0	10.0	10.0	10.0
Limestone	6.9	6.9	6.9	6.9
L-Lysine·HCl	5.8	3.8	1.8	-
DL-methionine	0.2	0.2	0.2	0.1
Vit. Mix[2]	1.2	1.2	1.2	1.2
Min. Mix[3]	1.2	1.2	1.2	1.2
Salt	1.0	1.0	1.0	1.0
Choline chloride, 25%	1.0	1.0	1.0	1.0
ZnO	1.0	1.0	1.0	1.0
Total	1,000.0	1,000.0	1,000.0	1,000.0
Chemical composition[4]				
ME, kcal/kg	3,265.7	3,265.2	3,265.0	3,265.9
CP, g/kg	209.0	209.0	209.0	209.0
Lys, g/kg	13.0	11.5	10.0	8.5
Met, g/kg	3.6	3.6	3.6	3.6
Ca, g/kg	7.0	7.0	7.0	7.0
Total P, g/kg	6.0	6.0	6.0	6.0

[1]Treatments: N + 0.15% (NRC requirement + 0.15% lysine), N (NRC requirement), N – 0.15% (NRC requirement – 0.15% lysine), N – 0.30% (NRC requirement – 0.30% lysine).

[2] Provided per kg of diet: 8,000 IU of vitamin A; 1,600 IU of vitamin D_3; 32 IU of vitamin E; 64 g of D-biotin; 3.2 mg of riboflavin; 8 mg of calcium-pantothenic acid; 16 mg of niacin; 12 g of vitamin B_{12}; 2.4 mg of vitamin K.

[3] Provided per kg of diet: 127.3 mg of Fe; 54.1 mg of Cu; 24.8 mg of Mn; 84.7 mg of Zn; 0.3 mg of Co; 0.3 mg of I; and 0.1 mg of Se.

[4] Calculated values.

Table 3. Dietary lysine levels on growth performance in weaning pigs[1]

Item	Treatments				SEM[2]	P-value	
	N+0.15%	N	N-0.15%	N-0.30%		Lin.	Quad.
Body weight, kg							
Initial	7.23	7.24	7.24	7.25	0.169	0.54	0.94
2nd wk	11.24	11.51	11.11	11.26	0.240	0.65	0.75
5th wk	20.38	21.11	19.87	18.41	0.395	0.01	0.02
ADG, g							
0-2nd wk	286.70	304.88	276.38	286.70	7.888	0.53	0.81
3rd-5th wk	434.82	457.28	417.10	340.45	11.609	0.01	0.01
Overall	375.57	396.32	360.81	318.95	8.563	0.01	0.03
ADFI, g							
0-2nd wk	380.04	392.59	365.09	383.39	6.330	0.82	0.62
3rd-5th wk	762.08	750.92	742.47	675.76	22.328	0.09	0.41
Overall	609.27	607.59	591.52	558.81	15.473	0.11	0.49
G/F ratio							
0-2nd wk	0.75	0.78	0.76	0.75	0.015	0.53	0.82
3rd-5th wk	0.57	0.63	0.57	0.51	0.014	0.02	0.02
Overall	0.62	0.66	0.61	0.58	0.011	0.02	0.02

[1]A total of 128 crossbred pigs with an average initial body weight 7.24 ± 0.03 kg.

[2]Standard error of mean.

Table 4. Dietary lysine levels on BUN in weaning pigs[1]

Item	Treatments				SEM[2]	P-value	
	N+0.15%	N	N-0.15%	N-0.30%		Lin.	Quad.
Initial	8.4	8.4	8.4	8.4	-	-	-
2nd wk	14.0	13.5	19.1	20.3	0.901	0.01	0.58
5th wk	15.1	16.9	16.7	27.4	0.930	0.01	0.01

[1]BUN, Blood urea nitrogen (mg/dL).

[2]Standard error of mean.

Table 5. Dietary lysine levels on nutrient digestibility in weaning pigs[1]

Item	Treatments				SEM[2]	P-value	
	N+0.15%	N	N-0.15%	N-0.30%		Lin.	Quad.
Nutrient digestibility, %							
Dry matter	89.80	89.11	88.94	89.13	0.145	0.07	0.10
Crude protein	88.50	87.16	87.49	86.79	0.368	0.19	0.62
Crude ash	58.97	56.10	51.57	55.55	1.014	0.06	0.06
Crude fat	45.91	44.59	45.58	43.18	1.002	0.43	0.95
Nitrogen retention, g/d							
N-intake	8.91	8.90	8.88	8.88	-	-	-
N-feces	1.02	1.14	1.11	1.17	0.032	0.19	0.66
N-urine	3.79	3.62	3.70	5.62	0.319	0.04	0.12
N-retention[3]	4.10	4.14	4.07	2.09	0.329	0.04	0.14

[1]A total of 24 crossbred pigs were used from an average initial body weight of 10.21±0.37kg.

[2]Standard error of mean.

[3]N retention = N intake (g) – Fecal N (g) – Urinary N (g).

Table 6. Dietary lysine levels on moisture content of feces in weaning pigs

Item	Treatments				SEM[1]	P-value	
	N+0.15%	N	N-0.15%	N-0.30%		Lin.	Quad.
Moisture content of feces, %							
2nd wk	77.44	75.07	74.63	75.64	0.441	0.05	0.07
5th wk	73.84	74.07	71.67	75.33	0.683	0.77	0.28

[1]Standard error of mean.

Table 7. Dietary lysine levels on fecal *E.coli* counts in weaning pigs

Item	Treatments				SEM[1]	P-value	
	N+0.15%	N	N-0.15%	N-0.30%		Lin.	Quad.
Fecal *E.coli* counts, $\log_{10}$ cfu/g							
Initial	4.70	-	-	-	-	-	-
2nd wk	5.24	5.45	5.54	5.37	0.136	0.91	0.67
5th wk	4.71	4.74	2.64	2.39	0.282	0.01	0.24

[1]Standard error of mean.

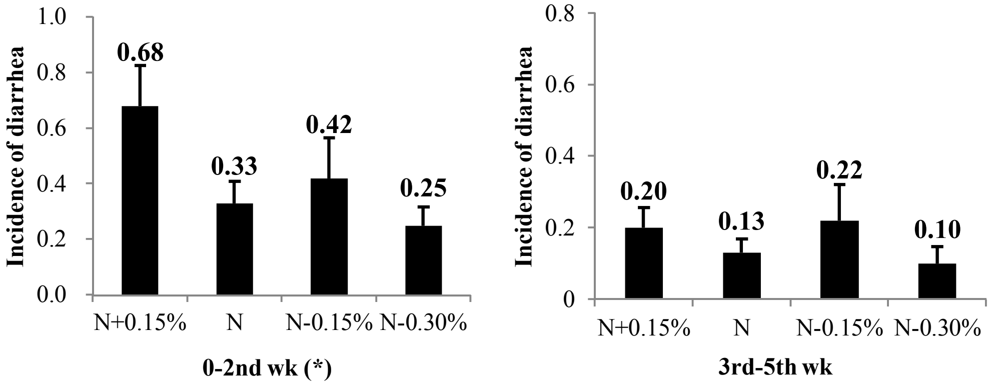

Figure 1. Dietary lysine levels on incidence of diarrhea in weaning pigs (*: linear effects by dietary lysine levels, P<0.05)

Chapter VI. Overall Conclusion

Evaluating nutrient requirements of pigs is the most important projection to decrease production cost and to improve swine productivity, but limited information is available or the consequences are inconsistent to evaluate the requirements of some nutrients. Therefore, three experiments were conducted to investigate 1) effects of various dietary energy levels of gestating diets in different season on reproductive performance and milk composition of sows, 2) effects of dietary methionine sources and supplementation levels on body conditions of lactating sows, and 3) effects of dietary lysine levels on growth performance, nutrient digestibility, blood profiles, fecal *E.coli* counts and incidence of diarrhea in weaning pigs.

Weight gain from day 0 to 110 of gestation were decreased in sows fed low energy diet (linear response, $P<0.05$) and sows mated during winter (season response, $P<0.01$). Then, both backfat thickness of day 110 of gestation (linear response, $P<0.05$) and backfat gains from day 0 to 110 of gestation (linear response, $P<0.01$) were subsequently shown to be increased when dietary energy level was increased. The changes of backfat thickness during lactation were higher when dietary energy level was increased (linear response, $P<0.01$). However, feeding diets containing different energy levels and mating sows in different seasons had no effects on litter weight, piglet weight and piglet weight gain during the whole lactating period, and then contents of fat, lactose, protein and total solid in sow milk were not changed by dietary treatments except the ratio of solid-not-fat at day 7 postpartum (season response, $P<0.05$) and the fatty acid profiles. The myristic acid at day 7 ($P<0.05$), 14 ($P<0.01$) and 21 ($P<0.05$) postpartum, the palmitic acid at day 14 postpartum ($P<0.01$), the palmitoleic acid at day 14 postpartum ($P<0.05$), the stearic acid at day 7 ($P<0.01$) and 14 ($P<0.01$) postpartum and the oleic acid at day

7 ($P<0.05$), 14 ($P<0.01$) and 21 ($P<0.05$) postpartum were increased when sows were mated during summer and palmitoleic acid at day 14 postpartum was decreased when dietary energy level was increased (linear response, $P<0.05$). A significant dietary energy level x season interaction was observed in the stearic acid content of milk at day 7.

Dietary methionine levels and sources are not associated significantly with the body weight and backfat thickness at day 21 postpartum, whereas the body weight changes in lactation (day 0 to 21) were changed by the supplementation level of L-methionine, resulting in linear and quadratic methionine level responses ($P<0.05$). Then, linear methionine level response by the supplementation of DL-methionine on this parameter was also detected ($P<0.05$). The WEI was decreased when L-methionine was supplemented to diets of lactating sows (sources response, $P<0.05$). Supplementing methionine to the diet of lactating sows had no effects on body weight and weight gain of nursery piglets, and then voluntary feed intake of lactating sows was also not affected. The colostrum and milk composition including milk fat, protein, lactose, total solid and solids-not-fat were not significantly different among dietary treatments and no differences were observed on plasma methionine level of piglets in day 21 of lactation. The sows fed diets containing 0.10% DL-methionine, 0.05% and 0.10% L-methionine showed higher plasma methionine levels compared to those fed control diet ($P<0.01$), resulting in linear effect by supplementing DL- and L-methionine ($P<0.01$). Then, the linear increase of methionine level in sow milk was found as dietary L-methionine level elevated ($P<0.05$).

Although feeding high lysine diets had no effects on feed consumption, average body weight at 5th wk and ADG of Phase II (3rd-5th wk) were decreased in pigs fed diets containing low level of dietary lysine, resulting in linear and quadratic response ($P<0.05$). Then, the G/F ratio was reduced as dietary level of lysine decreased with linear and quadratic response at Phase II and overall period ($P<0.05$). The pigs fed diets containing high level of dietary lysine showed lower BUN concentration compared to those fed diets containing low level of dietary lysine (linear responses at 2nd and 5th wks and quadratic response at 5th wk, $P<0.01$). There was no significant difference in apparent total tract digestibility of dry matter, crude protein, crude ash and crude fat, but linear increase of urinary nitrogen and linear decrease of nitrogen retention were detected as dietary lysine levels reduced (linear response, $P<0.05$). Feeding diets containing low level of dietary lysine had effects on reducing moisture content of feces at 2nd wk with linear response ($P<0.05$) and there was no significant effect on those results at 5th wk. Incidence of diarrhea at Phase I (0-2nd wk) and fecal *E.coli* counts at 5th wk were increased as dietary lysine levels increased, resulting in linear response of dietary lysine level ($P<0.05$).

Consequently, these strategies with dietary energy levels and season of mating, dietary lysine levels, and dietary methionine levels and sources in swine diets could improve swine productivity including physiological responses, WEI, availability of amino acids, and gut health.

Chapter VII. Summary in Korean

본 실험은 임신돈 사료 내 에너지 함량 및 번식계절이 번식성적, 유성분 및 포유자돈의 성적에 미치는 영향, 포유돈 사료 내 메치오닌 함량 및 공급원의 종류가 번식성적, 유성분 및 포유자돈의 성적에 미치는 영향 및 자돈 사료 내 라이신 함량이 성장성적, 영양소 소화율, 혈액 성상, 분성상 및 설사발생에 미치는 영향을 평가하기 위해 수행되었다.

Experiment I. Effects of Various Dietary Energy Levels of Gestating Diets in Different Season on Reproductive Performance and Milk Composition of Sows

본 실험은 임신돈 사료 내 에너지 함량 및 번식계절이 모돈의 번식성적, 유성분 및 포유자돈의 성적에 미치는 영향을 검증하기 위해 수행되었다. 총 36두의 F_1 임신모돈 (Yorkshire × Landrace)을 체중, 등지방 및 산차를 고려하여 2 x 4 요인설계 방법에 따라 배치하였으며, 계절에 따른 요인이 상대적으로 큰 영향을 미칠 것을 고려하여 통계 처리시 분할방법을 사용하였다. 요인은 번식계절과 (여름 및 겨울), 에너지 수준 (3,165, 3,265, 3,365 및 3,465 kcal of ME/kg)이었으며, 임신기 각각의 모돈에게 실험사료는 각각 2.4kg 씩 공급되었다. 실험 결과, 여름철에 종부한 경우와 사료 내 에너지 함량이 증가됨에 따라 모돈의 임신기 (0-110일) 체중 증체량이 증가되는 것으로 나타났으며 (linear energy response, $P<0.05$; season response, $P<0.01$), 110일령 등지방 및 임신기 등지방 증가량 (0-110일) 또한 사료 내 에너지 함량이 증가됨에 따라 함께 상승되는 것으로 나타났다 (linear response, P<0.05). 포유기 자돈의 성장 성적 및 모유 성상의 경우 처리구에 따른 효과가 나타나지 않았으나, 포유모돈 등지방 감소량의 경우 임신기 사료 내 에너지 수준이 높을수록 함께 상승되는 것으로 나타났다 (linear response, P<0.01). 모유 내 지방산 분석결과, 여름철에 종부를 수행한 경우 여러 항목에서 높은 수치를 나타냈으며 (분만 후 7일령 미리스트산, 스테아르산 및 올레산, 분만 후 14일령 미리스트산, 팔미트산, 팔미톨레산, 스테아르산 및 올레산, 분만 후 21일령 미리스트산 및 올레산; season response, P<0.05) 임신돈 사료 내 에너지 수준이 높을수록 분만 14일 후 팔미톨레산 함량을 감소시키는 것으로 나타났다 (linear response, P<0.05). 번식계절 및 임신돈 사료 내 에너지 함량에 따른 상호효과는 모든 실험 항목 중 유일하게 모유 내 지방산 함량 중 스테아르산에서만 발견되었다 (interaction effect, P<0.05). 결론적으로, 임신기 사료 내 에너지 함량을 증가시켜도 모돈의 번식성적 및 포유기 자돈의 성장성적에는 특별한 영향을 미치지 않는 것으로 나타났으며, 돈사 내 온도가 일정 수준 이상으로 유지될 경우 계절에 따라 해당 영양소 함량을 조절할 필요성도 없는 것으로 나타났다.

Experiment II. Effects of Dietary Methionine Sources and Supplementation Levels on Body Conditions of Lactating Sows

본 실험은 포유모돈 사료 내 메치오닌 공급원의 종류 및 첨가수준이 모돈의 생리적 반응, 혈액 성상, 유성분 및 포유자돈의 성장성적에 미치는 영향을 조사하기 위해 수행되었다. 35두의 F_1 임신모돈 (Yorkshire × Landrace)을 공시하여 분만 후에 체중, 등지방 및 산차를 고려하여 완전임

의배치법으로 5개의 처리구별로 배치하였다. 처리구는 메치오닌 공급원 (DL 및 L-메치오닌) 및 첨가수준 (0.05% 및 0.10%) 에 따라 대조구를 포함하여 총 5가지로 구분되었으며, 각각의 실험사료는 포유기간 동안 무제한 급여로 제공되었다. 실험 결과, 포유모돈의 체중 및 등지방의 경우 처리구에 따른 유의적인 차이가 나타나지 않았다. 반면에, 포유기 체중 감소량의 경우 대조구에 비해 DL-메치오닌 0.10% 첨가구, L-메치오닌 0.05% 첨가구 및 L-메치오닌 0.10% 첨가구를 급여한 경우 감소되는 것으로 나타났으며, 이유 후 재귀발정일의 경우 L-메치오닌을 첨가한 사료를 급여하였을 때 DL-메치오닌을 급여하였을 때보다 유의적으로 개선되는 것으로 나타났다 (source response, $P<0.05$). 또한 포유자돈의 성장성적 및 포유기 모돈의 사료 섭취량에서 처리구에 따른 유의적인 차이가 나타나지 않았으며, 이러한 경향은 포유기 모유 내 지방, 단백질, 유당, 총 고형물 및 비지방 고형물 함량을 분석한 결과에서도 동일하게 유지되었다. 서로 다른 메치오닌 공급원을 여러 수준으로 급여하였을 때 모돈의 포유 21일령 혈액 내 메치오닌 함량에 미치는 영향을 분석한 결과, 0.10% DL-메치오닌, 0.05% L-메치오닌 및 0.10% L-메치오닌 첨가구를 모돈에게 급여한 경우 대조구에 비해 높은 수치를 나타냈으며 ($P<0.01$), 모유 내 메치오닌 함량의 경우 L-메치오닌 첨가수준이 증가할수록 높아지는 경향을 나타냈다 (linear response, $P<0.05$). 결론적으로, 메치오닌 첨가수준 및 공급원은 포유 모돈의 모유 성상, 사료섭취량 및 자돈의 성장성적에 특별한 영향을 미치지 않는 것으로 나타났으며, 이유 후 재귀발정일 및 포유기 모돈의 체중 감소량의 경우 L-메치오닌을 급여하였을 때 DL-메치오닌을 급여한 경우보다 개선되는 결과를 나타냈다.

Experiment III. Effects of Dietary Lysine Levels on Growth Performance, Nutrient Digestibility, Blood profiles, Fecal *E.coli* Counts and Incidence of Diarrhea in Weaning Pigs

본 실험은 사료 내 라이신 수준이 자돈의 성장성적, 영양소 소화율, 혈액성상, 분성상 및 설사발생에 미치는 영향을 규명하기 위하여 수행되었다. 28 ± 2일령에 이유된 평균 체중 7.24 ± 0.03 kg의 3원 교잡종 ([Yorkshire × Landrace]) × Duroc) 자돈 128두를 공시하였으며, 전체 4처리 8반복, 반복당 4두씩 성별과 체중에 따라 난괴법 (RCBD; Randomized Complete Block Design)으로 배치하였다. 실험의 처리구는 1) N + 0.15% (NRC 요구량 + 0.15% 라이신 첨가), 2) N (NRC 요구량 라이신 첨가), 3) N - 0.15% (NRC 요구량 - 0.15% 라이신 첨가) 및 4) N - 0.30% (NRC 요구량 - 0.30% 라이신 첨가)로 나뉘었으며, 육성기와 비육기 사료 내 에너지 함량은 동일하게 3,265 kcal of ME/kg으로, 단백질 함량은 각각 23.7% (Phase I, 0-2주)와 20.9% (Phase II, 3-5주)로 설계하였다. 사양실험 결과, 사료 내 라이신 함량을 증가시킬 경우 3-5주 사이의 ADG 및 사료효율이 증가되는 것으로 나타났으며 (linear response, P<0.05; quadratic response, P<0.05), Phase I 기간에는 모든 관련 항목에 특별한 영향을 미치지 않는 것으로 나타났다. 자돈을 대상으로 시행된 영양소소화율 실험결과, 건물, 조단백, 조지방 및 조회분 소화율의 경우 사료 내 라이신 수준에 따른 영향이 나타나지 않았으며, 2주차 및 5주차 BUN 분석결과 라이신 수준을 향상시킬수록 유의적으로 낮은 수치를 나타내 개선된 아미노산 이용효율을 보였다 (0-2주차: linear response, P<0.01; 3-5주차: quadratic response, P<0.01). 질소축적율 분석 실험을 수행한 결과, 사료 내 라이신 수준이 증가될수록 감소된 요중 질소 함량 및 증가된 질소축적

율을 나타냈다 (linear response, $P<0.05$). 또한, 높은 수준의 라이신을 포함한 사료를 급여한 자돈의 경우 2주차 분내 수분 함량이 증가되는 것으로 나타났으며 (linear response, $P<0.05$), 높은 5주차 분내 대장균수 및 0-2주차 설사발생율을 보였다. 결론적으로, 20.9%의 조단백질을 포함한 사료 내 라이신을 1.15%/kg 수준으로 설정하고, 11-20 kg의 자돈에게 급여할 경우 증체량 및 사료효율이 개선되는 것으로 나타났다. 또한, 사료 내 라이신 수준을 향상시킬 경우 BUN 및 요중 질소 함량을 떨어뜨리는 것으로 나타났으며, 과도하게 높은 수준은 설사발생율 및 분 내 대장균수를 증가시킬 수 있을 것으로 사료된다.

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