A Dissertation

for the Degree of Doctor of Philosophy

Effects of Alternative Nutritional Manipulation on Physiology and Productivity of Weaning Pigs and Sows

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Effects of Alternative Nutritional Manipulation on Physiology and Productivity of Weaning Pigs and Sows

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Overall Summary

Effects of Alternative Nutritional Manipulation on Physiology and Productivity of Weaning Pigs and Sows

The objectives of these experiments were 1) to investigate the effect of benzoic acid supplementation on the performance, nutrient digestibility, blood profile and ammonia gas emission in weaning and growing pigs, 2) to investigate the influence of different energy and dietary lysine levels in gestation diets on reproductive performance, blood composition and growth of gilts and 3) to investigate the effects of different levels of fat supplementation during gestation on reproductive performance, milk composition and their progeny performance of sows.

Experiment I. Effect of VevoVitall® Supplementation on the Performance, Nutrient Digestibility, Blood Profile and Ammonia Gas Emission in Weaning and Growing Pigs

The experiment was conducted to investigate whether VevoVitall® supplementation provided by DSM Nutrition Korea Ltd. was suitable as an antibiotic alternative and would produce a synergistic effect with antibiotics on growth performance, urine pH, blood profile, nutrient digestibility and ammonia emission in weaning and growing pigs. The experiment consisted of 4 treatments: 1) Ncon (negative control), basal diet (corn-soy bean meal based); 2) Pcon (positive control), basal diet +Colistin sulfate0.12%; 3) NeVe, basal diet + VevoVitall® 0.5%; and 4) PoVe, basal diet + Colistin sulfate 0.12% + VevoVitall® 0.5%. Treatments were applied during weaning (5 weeks) and growing (6 weeks) periods. Crossbred pigs (n = 128; [Landrace x Yorkshire] x Duroc) weaned at 24 ± 3d of age were used. After weaning, body weight (BW), average daily gain (ADG) and average daily

feed intake (ADFI) were higher in treatment Pcon, NeVe and PoVe than in Ncon ($P<0.05$). No difference was observed among NeVe and PoVe with added VevoVitall® supplementation to diet and Pcon. Growth performance during the weaning phase was directly influenced on growth of growing phase. During the growing phase, there was no significant difference in ADG; however, Pcon, NeVe, and PoVe tended to show a more positive effect than Ncon. In this experiment, the results did not reveal an interaction or synergistic effect between antibiotic and VevoVitall® supplementation; however, the growth performance of PoVe was greater than that of other treatments. No significant differences were found in nutrient digestibility, blood urea, nitrogen concentration, and nitrogen retention among dietary treatments. In case of urine pH trial, urine pH was lower when VevoVitall® was supplemented (NeVe and PoVe) in weaning pig's diet($P=0.069$). Also, in growing phase, treatment PoVe (antibiotic with VevoVitall® supplementation) showed lower urine pH ($P=0.059$). The pH in feces tended to be loweredby 9.01% and 4.05% respectively when pigs were fed diets supplemented with benzoic acid (NeVe and PoVe), and ammonia gas emission was also reduced by benzoic acid supplementation although the difference was not statistically significant. White blood cell, red blood cell, lymphocyte, total protein and albumin level were determined if there were immune responses detected in blood related to VevoVitall® supplementation. However, no significant differences were observed among all treatments. In conclusion, the results from this experiment suggested that VevoVitall® supplementation in weaning pig's diet had a significant influence on growth performance of weaning pigs.

Experiment II. Influence of different energy and dietary lysine levels in gestation diets on reproductive performance, blood profiles and growth of gilts

The aim of this experiment was to determine the influence of different energy andlysine levels of gestating diet on physiological parameters and

reproductive performance of primiparous sows. A total of 28 F1 gilts (Yorkshire x Landrace) were allotted to a 2 x 2 factorial arrangement of treatments in a completely randomized design with 7 replicates. Factors evaluated were energy levels (3,050 kcal of ME/kg or 3,140 kcal of ME/kg) and dietary lysine levels (0.64% or 0.74%). The each experimental diet was provided to gilts of 2.1 kg/d during gestation period. Total lysine intake of each treatment gestating gilts was 13.4, 15.5, 13.8 and 15.9 g/day, respectively. There were no treatment effects on the body weight changes from breeding day to 90 d of gestation period ; however, body weight changes during the whole gestation period (0~110d) was affected by dietary energy and lysine levels. When gilts were fed high energy (3,140 kcal of ME/kg) and high dietary lysine (0.74%) diet showed higher body weight gain (increasing 65.75 kg) than other treatments ($p<0.05$). Backfat thickness of primiparous for gestation period was not affected by energy lever or level of dietary lysine ($P>0.10$). Higher lysine level (0.74%) in gestation diet did not show more backfat gain of gilts than low lysine level (0.64%). The number of piglet per litter and litter birth weight were numerically higher when gilts were fed low energy intake (6,405 kcal of ME/d) and 0.64 % lysine level diet during gestation period even though it didn't show significant difference among treatments. Also, higher energy (6,594 kcal of ME/d) or higher lysine (15.5g lysine/d) in gestation diet did not show any positive reproductive performance of gilts. The level of plasma glucose and insulin did not change with high level of energy intake or lysine intake during gestation period. The concentration of blood urea nitrogen at 35 d, 70 d and 90 d in gestation period numerically increased as the dietary lysine level increased ($p<0.05$). Consequently, these results demonstrated that the energy level and dietary lysine level more than 6,400 kcal of ME/day and 13.4 g/day would be enough for gilts to meet theirrequirements without negative effects on growth performance and reproductive performance.

Experiment III. Effects of Different Levels of Fat Supplementation during Gestation Period on Reproductive Performance, Milk

Composition and their Progeny Performance of Sows

The aim of this experiment was to determine the influence of different levels of fat during gestation period on reproductive performance of sows. A total of 41 F1 multiparous sows (Yorkshire x Landrace) were allotted to 4 treatments by completely randomized design. During gestation, sows were fed different treatments containing either 1, 2, 3 or 4% of soybean oil whereas all sows were fed the same diet containing 1% soybean oil during lactation. There were no significant differences in body weight and backfat thickness of sows at 110 d postcoitum and 21 d postpartum regardless of dietary fat levels during gestation. Although the change in body weight of gestating sows were similar among treatment groups, the lowest backfat thickness change at 110d postcoitum was observed when sows were fed 3% soybean oil treatment diet ($P<0.05$). The litter size, total born live, birth weight and weight gain of nursing piglets were not influenced by the inclusion level of soybean oil during gestation. Fat content in colostrum had no response to increased fat level of gestation diet. The mortality of nursing pigs tended to be higher as dietary fat level was increased. No effects were observed in weaning to estrus interval by the different supplementation levels of soybean oil. In economic analysis of gestation feed, the difference price of total gestation feed cost (per sow) is 7.92$ between 1% fat level and 4% fat level of gestation diet. These results demonstrated that higher levels of fat in sow′s diet did not show any beneficial effect on reproductive performance of sows. Moreover fat concentration of colosturm was not increased by dietary fat supplementation in gestating sow’s diet.

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List of Abbreviation

ADFI	average daily feed intake
ADG	average daily gain
BFT	backfat thickness
BUN	blood urea nitrogen
BW	body weight
$BW^{0.75}$	Metabolic body weight
CP	crude protein
DE	digestible energy
FFA	Free fatty acid
FTA	free trade agreement
GE	gross energy
GI	gastrointestinal tract
GLM	general linear model
ME	metaboilzable energy
MSY	market pigs per sow per year
NE	net energy
NSP	non-starch polysaccharide
SBM	soybean meal
VFA	volatile fatty acid
WEI	weaning to estrus interval

Chapter I. General Introduction

Productive capacity of swine industry in Korea is remarkably lower than those of industry in Europe countries despite steadily increasing every year. For instance, market pigs per sow per year (MSY) in Korea was approximately 17.6 (Korea pork producers association, 2013), but those in Netherlands, Denmark, France and Ireland were 26.97, 26.93, 25.19 and 24.11, respectively (INTERPIG, 2012). In addition, litters per sow per year in Netherlands, Denmark, France and Ireland were 2.38, 2.26, 2.34 and 2.31, respectively which is higher than those score recorded 2.18 in Korea. There are several reasons for low productivity in Korea, such as lower litter size, higher mortality rate of weaned pig and lower number of litters per sow per year.

Domestic swine farms are suffering a setback in their performances due to lower productivity and a soaring production costs. The largest part of the production cost is taken up by a feed cost and livestock cost. Therefore, it is important to enhance feed efficiency for reducing feed cost. There are many methods in which producer can increase feed efficiency nutritionally, most of which affect directly to the improvement of overall gut health of the animal that ultimately leads to improving feed conversion. Some of those include antibiotic growth promoters, gut acidifiers, enzymes and probiotics. AGPs show the highest benefit in enhancing average daily gain and feed conversion but organic acids are one of the alternatives and mixed combinations of AGP alternatives can come close to the performance response seen with AGPs (Dansk Slagterier, 2001). Organic acids such as propionic, formic, citric, lactic and fumaric have used the swine diet, especially piglet diets. These may help to counter the aptness of pH rise in the stomach which can occur when piglets are stressed. Diet organic acids may help the gut environment and through that assistance improve feed intake and decrease the

incidence of diarrhea post weaning. In some studies, benzoic acid has been shown to increase daily weight gain and to improve feed per gain ratio in piglets and growing-finishing pigs. In addition, some of experiments about benzoic acid in diet have been shown reducing urinary pH. This reduction of urinary pH can reduce ammonia emission, which is of great importance in swine farm. However, results of growth performance from *in vivo* studies are less consistent. There is a few experimental evidence giving clear results about the mode of action.

The better the approach of fit between swine nutrient requirement and diet nutrient composition, the greater will be the efficiency of conversion of diet into swine product and the more will improve performance be achieved. Thus many swine nutritionist have research the application of nutrient requirement for the last several decades. However, those of research have to be still necessary because modern pigs have been changed genetically to enhance for higher productivity and also requirements of modern pigs have been changed. In case of sows, during pregnancy, nutrient requirements of modern sows for maintenance, uterine and maternal growth should be higher than in the past due to the improved prolificacy and the heavier mature body weight.

To investigate the effects of dietary fat in gestation diet, a considerable amount of experiments had been conducted. Some studies suggested that an increase body lipid content in piglets at weaning in association with increased milk fat content, thus indicating that most of the lipids from the milk are deposited in the piglet's body. Several nutritionists indicated that adding fat to sow diet increases the fat content of the colostrums. Increased fat content in colostrums may increase piglet survival. However, in some studies, the effects on sow and litter performance are variable. Also, Dietary fat level was too high in those of experiments which have shown positive effects. Therefore, this study evaluated the effects of economically optimum levels dietary fat in diet of gestation sow.

Consequently, to overcome this challenges at swine industry, three experiments were conducted to investigate 1) the effect of benzoic acid supplementation on the performance, nutrient digestibility, blood profile and ammonia gas emission in weaning and growing pigs, 2) the influence of different energy and dietary lysine levels in gestation diets on reproductive performance, blood composition and growth of gilts and 3) the effects of different levels of fat supplementation during gestation on reproductive performance, milk composition and their progeny performance of sows.

Chapter II. Literature Review

1. Feed Additives for young pigs

1.1 General Approaches

Additives may be defined as ingredients used for purpose other than directly supplying essential nutrients and energy. Additives are used to enhance performance or prevent loss of performance. Additives may be classified as 'zootechnical', in that they interact with the animal, or 'technical', in that they interact mainly with feed quality. Most countries have their own definitions and classifications of additives and often regulate their application to some extent.

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.

1.2 Antibiotics

Antibiotics are chemical compounds that, when given in small amounts, prevent the growth of bacteria. Those things are produced by other microorganisms, e.g. fungi, and are also synthesized in the laboratory. They are used at therapeutic levels, by food or water or injections, to treat diseases caused by unknown bacteria. In addition, sub-therapeutic levels of antibiotics are added to the food to enhance the growth rate. The various antibiotics act in different ways to reduce the numbers of specific bacteria in the gut, and thereby increase the efficiency of nutrient utilization.

Feed-grade antimicrobial agents are well-established additives that enhance feed efficiency and control the gastrointestinal microflora, resulting in improved growth performance (Cromwell, 2002). In young pigs, the magnitude of response depends on the level of hygiene in facilities and the health status of pigs. Under most commercial farm conditions, it is expected that antimicrobial agents increase growth performance by 5-15% over non-supplemented controls. Nevertheless, the use of feed-grade antimicrobial agents is declining worldwide due to concerns regarding increasing bacterial resistance to these compounds (Goransson, 2001). Effective alternatives to young pigs are currently in high demand.

1.3 Enzymes

Enzymes are used to enhance digestion of specific nutrients (starch, proteins and lipids) or enable, at least partially; digestion of otherwise indigestible compounds (mainly non-starch polysaccharides and phytate). In Table 1, several enzyme preparations are presented along with their target compounds and relative efficacy. In young pigs, the ability to digest starch, proteins, and lipids is not enhanced by dietary supplementation of corresponding enzymes, at least to the point to justify their cost. Research has been rather inadequate and confusing at best. Most authorities agree that young pigs fed high-quality diets are not likely to benefit markedly from such enzymes (Partridge, 2001).

Supplementation of pig feeds improves ileal and whole tract digestibility of nutrients. In breaking down the b-glucans, the enzymes reduce the viscosity of the digesta, thus allowing better movement of endogenous enzymes through the mass and more efficient digestion and absorption of nutrients. It is still not clear whether viscosity per se is responsible for the effects or whether it is an indicator of conditions pertaining in the gut lumen that cause the problems. Early-weaned pigs have limited amylase, protease and lipase activity, and enhancement of the extent of digestion of nutrients would improve performance and reduce the incidence of the diarrhea that results from undigested nutrients reaching the hind gut and being fermented by bacteria.

In order to be effective when incorporated into the pig's diet, enzymes must survive storage at ambient temperature, the manufacturing process (heating and pelleting) and wide fluctuations in pH in the gut, be resistant to intestinal proteases, and have specific activity on feed components in the upper digestive tract. The enzyme should be selected on the basis of its target substrate.

Table 1. Description of enzymes available for piglet feeds [a] (Mavromichalis, 2006)

Enzyme	Main target	Ingredients	Effectiveness in piglets
Amylase	Starch	All cereals	Small
b-Glucanase	NSP[b]	Barley, wheat, rye, triticale	Medium
Lipase	Lipids	Lipids and oil seeds	Trivial
Phytase	Phytate	All plant-based ingredients	High for P/Ca
Protease	Proteins	Plant-derived ingredients	Unclear
Xylanase	NSP[b]	Wheat, triticale, barley, rye	Depends on cereal quality

[a] Consult manufacturer's directions before using any specific product.

[b] Non-starch polysaccharides (NSP) like b-glucans and arabinoxylans.

1.4 Plant extracts

Plant extracts from spices and herbs have a huge history in human traditional medicine, and those things have emerged recently as novel additives for antibiotic free diets (Kamel, 2000). The modes of action are usually unknown, but claims exist for enhanced appetite and digestion, along with stimulation of the immune system and control of the gastrointestinal microflora (Gill, 1999). Table shows the major extracts used nowadays in most commercial preparations. Research on the efficacy of plant extracts in piglet is rather limited and largely represented.

Table 2. Common plant extracts used in diets for piglets[a]. (Kamel, 2000)

Source	Active compound(s)	Properties
Cinnamon	Cinnamaldehyde	Appetite and digestion stimulant, antiseptic
Oregano	Carvacrol	Appetite stimulant, antiseptic
Clove	Eugenol	Appetite and digestion stimulant, antiseptic
Garlic	Allicin	Digestion stimulant, antiseptic
Capscium	Capsaicin	Anti-diarrheic, anti-inflammatory
Thyme	Thymol	Appetite stimulant, antiseptic, antioxidant

[a] Consult manufacturer's directions before using any specific product.

1.5 Minerals

Poulsen (1989) mentioned a growth performance response of weaning pigs to supplementation of the diet with 3,000 ppm of zinc from zinc oxide. Several papers have confirmed this response (Hahn and Baker, 1993; Hill et al., 1996; Smith et al., 1997). The response to zinc did not seem to be effective additive to that for antimicrobials unlike copper. In a couple of studies, Mahan et al.(2000) reported that weaning pigs fed 3,000 ppm zinc with or without Carbadox responded to zinc supplementation, but the increase in growth performance was not additive to that observed with Carbadox supplementation.

Supplementation of weaning pig diets with copper has consistently improved growth performance of pig (Braude, 1967; Cromwell et al., 1989). Moreover, the improvement in piglet performance with supplementation of copper is independent and additive to that for antibiotics (Roof and Mahan, 1982; Cromwell, 1991). Because both copper and zinc improve a growth performance in weaning pigs, there has been interest in determining if the response to zinc and copper is additive. Hill et al.(1996) and Smith et al.(1997) showed an improvement in growth performance of weaning pigs fed zinc and copper, but the response were not additive.

1.6 Organic Acids

Studies on the organic acids in diets to increase gastric acidification in young pigs have established a basis for the use of acids in weaning pig diets. The digestive systems of the early weaned pig are not sufficiently developed to handle the conversion from diets based on milk proteins to those based on plant protein. Some problem could be the lack of protein denaturation, which could be a consequence of a higher gastric pH than the acidic pH values (pH 2.0 to 3.5) of mature pigs (Kidder and Manners, 1978). Acid secretion is not enough to reach appreciable levels until day 21 and 28 postweaning (Cranwell and Moughan, 1989). Easter (1988) mentioned the strategies used by nursery pig to overcome the

limitations of gastric acid secretion. These include the transition of lactose to lactic acid by lactobacilli bacteria in the stomach and the frequent ingestion of small meals, thereby decreasing the demand for high levels of acid secretion. The collapse to maintain a low gastric pH has major implications for the performance of early weaning pigs, furthermore protein denaturation. These cause decreased digestion by pepsin, handle of gastric emptying, and bacteriocidal effects on certain microorganisms (Ravindran and Kornegay, 1993). However, this suggests that reducing pH does not seem to be one of the main effects of acidification (Sweet et al., 1990; Risley et al., 1992; Roth et al., 1992; Burnell et al., 1988; Sutton et al., 1989). Despite the insufficient evidence of a direct effect of dietary acidifiers on pH, acidifiers have been shown to have an effect of controlling digestive scouring (White et al., 1969) and decreasing the associated coliform burden along the gastrointestinal tract (Cole et al., 1968; Thomlinson and Lawrence, 1981; Bolduan et al., 1988; Bokori et al., 1989; Mathew et al., 1991; Eckel et al., 1992; Johnson, 1992). Mathew et al. (1991) showed that lactobacilli population was decreased to almost zero within few days of weaning whereas the coliform population increased. This change was associated with an increased pH in the ileal contents.

Although efficacy of acidifiers has been demonstrated in numerous studies, responses have been inconsistent with all types of acidifiers. Ravindran and Kornegay(1993) published a review of articles summarizing the effects of numerous acidifiers on pig performance. Those results of study suggested that variables that influence efficacy of acidifiers include type and dosage of acidifier, type of diet, age of pigs, and existing environmental conditions.

Citric acid is a rather complex acid with three carboxylic groups. At room temperature it is present as white and odorless crystals with a sour taste. Citric acid is mainly present in fruits and vegetables, especially in citrus fruits. As one of the first intermediate in the citric acid cycle, citric acid and its salts can be found in

almost all organisms. Many microorganisms are adapted to this acid because of its importance in the metabolism. For this reason citric acid is not such an efficient antimicrobial agent as other acids (Partanen and Mroz 1999). In spite of this, the addition of citric acid affected performance of piglets positively (Tsiloyiannis et al. 2001a; Giesting and Easter 1985; Radcliffe et al. 1998) and chickens (Liem et al. 2008; Boling et al. 2000; Rafacz-Livingston et al. 2005). To what amount these effects are based on antimicrobial activity and how much they are caused by the digestible energy content of 10.2 MJ/kg or by boosting the citric acid cycle is unclear. Apart from its growth enhancing properties citric acid can also increase the utilization of phytate P in chickens (Ebrahimnezhad et al. 2008; Boling et al. 2000; Liem et al. 2008).

Formic acid, a colourless liquid with a pungent odour, is the simplest of the organic acids. It can be found in insects and plants where it mainly serves as defending agent. Formate is an integral part of the metabolism, especially in the transfer of 1-C intermediates and in the synthesis of purines (Partanen and Mroz 1999). In animal nutrition formic acid is mostly used as formate, as the salt is less corrosive and less toxic than the free acid. There is an abundance of articles in piglets and growing-finishing pigs describing growth enhancing and antimicrobial effects of formic acid and its salts (Bolduan et al. 1988a; Overland et al. 2000; Eidelsburger et al. 1992c; Partanen et al. 2001; Ettle et al. 2004; Eidelsburger et al. 2005; Eisemann and van Heugten 2007).

1.6.1 Benzoic Acid

Compared to other organic acids, benzoic acid seems to fulfil the tasks of replacing antibiotics as growth promoters very well (Den Brok et al. 1999; Canibe et al. 2001). Another advantage of benzoic acid is its potential to reduce ammonia (NH3) emissions from excrements. This is of particular importance as the negative

effects of NH3 on the environment can be reduced. Furthermore, NH3 is an olfactory nuisance and it is known to cause respiratory problems in humans and animals (Ferket et al. 2002). Most of the NH3 in pig waste is a degradation product from urea produced by the bacterial enzyme urease found in feces (van Kempen 2001). In contrast to other organic acids, benzoic acid is metabolized in the liver to hippuric acid which is excreted by the urinary pathway (Bridges et al. 1970). The concentration of hippuric acid in the urine lowers urinary pH and thus the activity of urease which depends on pH. Benzoic acid is found in fruits and berries and is used as a preservative in human nutrition. Since May 2003 benzoic acid has been provisionally registered in the European Union as additive in pig nutrition (EC 877/2003).

Benzoic acid is the simplest of the aromatic carboxylic acids and forms colourless to white crystals. It occurs in different resins, in fruits and berries, especially from the genus Vaccinium, but also in milk and milk products and animal tissue and gland secretions (CICA 2000). This acid has been used for preservation in nutrition of human for a long time, but it was only used to pig nutrition in 2003. In pig nutrition, benzoic acid has been shown to increase body weight gain and to improve feed efficiency (van der Peet-Schwering et al. 1999b; Maribo et al. 2000; Dierick et al. 2004; Guggenbuhl et al. 2007b) in piglets and growing-finishing pigs. In *in vitro* researches benzoic acid showed the effect of reducing overall caecal microflora (Biagi and Piva 2005) and also in imposing negative effects on coliforms as well as on lactic acid bacteria (Knarreborg et al. 2002). Results from *in vivo* researches are less consistent. Some articles agree with the *in vitro* results (Maribo et al. 2000), others show counts of lactic acid bacteria which could increase or decrease depending on the amount of benzoic acid in the diet (Kluge et al. 2006). Finally, some articles describe increasing numbers of E. coli (Dierick et al. 2004; Torrallardona et al. 2007). Contradictory experimental

results can be explained by the effects depend on the examined section of the gastrointestinal tract. The effects could be oppositional depending on the gastrointestinal segments examined.

Beside its antimicrobial effects, benzoic acid can also reduce urinary pH (Kluge et al. 2006; van der Peet-Schwering et al. 1999b; Plitzner et al. 2006). After absorption from the small intestine, the benzoic acids are transported to the liver where it conjugates with the amino acid glycine to hippuric acid. In this form benzoic acid is then excreted to almost up to 90% within 24 h via the urine (Bridges et al. 1970). This reduction of urinary pH can markedly reduce ammonia emission (Mroz et al. 2000; Hansen et al. 2007), which is of problem in areas with pig production.

Benzoic acid is a phenyl formic acid which belongs to the group of weak organic acids. As it was to be expected, the addition of benzoic acid caused a dosedependent reduction of the dietary pH and buffering capacity. It has been shown that benzoic acid has antimicrobial properties, mainly because of its inhibitory effect on several microbial enzymes, in particular a-ketoglutaric acid dehydrogenase and succinic acid dehydrogenase (Bosund, 1962). A growth inhibitory effect of benzoic acid has been reported particularly in yeasts while a weak effect in this respect was observed in bacteria (Uraih and Chipley, 1976).

Benzoic acid resembles formic acid as regards its effect on microbial growth. This was why the effects of benzoic acid were compared in the performance trial with those of potassium diformate. Several studies have shown that potassium diformate enhances the growth performance of piglets (Paulicks et al., 1996; Kirchgessner et al., 1997; Roth et al., 1998; Canibe et al., 2001). As reported by Paulicks et al.(1996), supplementation of piglet diets with potassium diformate at 18 g/kg of diet increased mean body weight gain and improved feed conversion ratio of piglets by 14% and 7% respectively. Supplementation of the

diet with benzoic acid at 10 g/kg resulted in comparable improvements in mean body weight gain and feed conversion ratio.

Kirchgessner and Roth (1988) suggested that the effect of organic acids on the growth performance of piglets is at least partly associated with a reduction of dietary pH and dietary buffering capacity, which reduces the pH in the stomach, enhances proteolytic digestion and controls the growth of pathogenic bacteria. Non-dissociated organic acids can passively diffuse through the bacterial cell wall, dissociate themselves when the pH is above the pKa and cause the internal pH to fall, which is incompatible with certain categories of bacteria that do not tolerate a steep gradient in the trans membranous pH. In this case, a resistance mechanism that reacts to this type of cellular stress will turn on and protons will be pumped out of the bacteria by an ATPase pump, which expends energy and exhausts the bacterium. The anions will accumulate in the cell, modify the internal osmotic pressure and become toxic to the bacterium (cessation of glycolysis and of nucleic acid synthesis, blocked enzymatic reactions, disturbance of membranous transport, etc.) (Jensen, 2001). Benzoic acid presumably acted in this manner as a non dissociated acid. The finding that supplementation of benzoic acid does not alter the pH in the stomach and small intestine is consistent with a recent study performed in piglets (National Committee for Pig Production, 2000). In that study even 20 g benzoic acid per kilogram of diet did not significantly reduce the pH along the entire gastrointestinal tract of piglets but caused a large bacterial reduction (lactic acid bacteria and lactobacilli) throughout the gastrointestinal tract.

1.6.2 Mode of Action

Even though the exact mode of action is not yet clear, the organic acid has been commercially used in swine diet. The potential of organic acids to change from the undissociated (RCOOH) to the dissociated (RCOO- + H+) form and vice

versa seems to be a crucial element. According to Lambert and Stratford (1999) a key point in the equilibrium among the two forms is the pKa, defined as the "pH value at which there exist equal proportions of molecular acid and charged anions". This makes organic acids the more potent antimicrobials the lower the pH is (Lambert and Stratford 1999). The undissociated acid, which dominates in an acidic environment, is lipophilic and can cross the bacterial or fungal cell membrane. Once inside the microbial cell the acid dissociates as the cellular pH is close to neutral (Figure 1.). The increase of H+ leads to a decrease in pH forcing the cell to actively remove the protons from the cytoplasma. This is an ATP dependent and thus energy consuming mechanism making the bacterial or fungal cell less competitive or even killing it (Brul and Coote 1999; Ricke 2003; Theron and Lues 2007). Furthermore, the decrease in cytoplasmic pH hampers enzymatic reactions and nutrient transport systems (Cherrington et al. 1991).

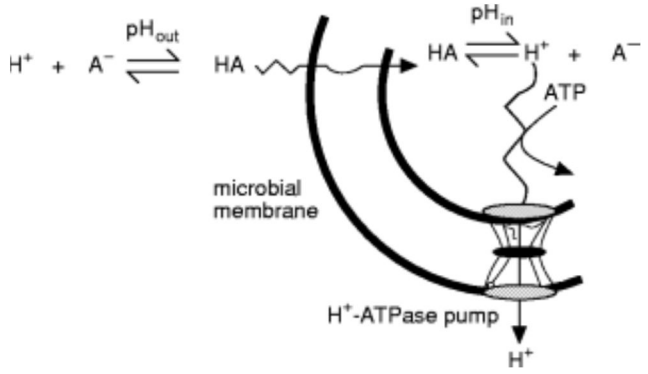

Figure 1. Predicted cytoplasmic weak-acid/anion equilibrium (Lambert and Stratford 1999)

On the other hand the accumulation of polar anions is toxic for the cell and causes an osmotic pressure (Russell 1992; Roe et al. 1998). Other possible modes of action include membrane disruption and inhibition of bacterial

metabolism as it was reviewed by Brul and Coote (1999). Under these conditions and in combination with reduced pH in the gastrointestinal tract, beneficial microorganisms such as lactic acid bacteria are favoured over potential pathogens such as E. coli and Salmonella spp. (Mathew et al. 1991; Overland et al. 2000; Knarreborg et al. 2002). Indirect effects of organic acids include reduction of gastric emptying rate, increased intestinal enzyme secretion and activity, additional energy source, improved protein digestion and increased mineral availability (Ravindran and Kornegay 1993; Partanen and Mroz 1999; Partanen 2001; Kim et al. 2005).

So far there has been no report on bacterial resistance to organic acids but acid induced protection and adaptation systems do exist (Lin et al. 1996; Guilfoyle and Hirshfield 1996; Lambert et al. 1997; Lambert and Stratford 1999; Brul and Coote 1999; Piper et al. 2001; Richard and Foster 2003; Ricke 2003). It has been shown several times that organic acids act the better, the younger the animal is (Gabert and Sauer 1994; Roth and Kirchgessner 1998). This age dependent effect was explained by the fact that the immature intestinal microflora is more susceptible to acid induced changes than the mature microbial community found in older animals (Jensen 1998; Partanen and Mroz 1999). How much a possible adaptation of the microorganisms to organic acids is involved in this observation is unclear.

Other additives than organic acids also proposed to be an alternative for AGP are prebiotics (oligosaccharides), probiotics (mainly Lactobacilli), herbs and essential oils (Wenk 2000). Comparing the alternatives it seems that organic acids are the most promising substances – beside strict management and hygiene measurements (Adjiri-Awere and Van Lunen 2005) – to compensate for the ban on AGP (Jensen 1998; Metzler et al. 2005). However, results depend on diet type, diet composition, dietary buffering capacity, age and health of the animal as well as on

the length of the feeding period (Ravindran and Kornegay 1993; Gabert and Sauer 1994).

2. Dietary Energy and Lysine in Gestating Sows

2.1 Energy systems of Gestating Sows

The metabolizable energy (ME) of carbohydrate and lipid may be taken as 1.0 of the same as the digestible energy (DE). This is not the case for fibre, where small gas losses and reduced efficiencies of utilization occur when volatile fatty acids are yielded from microbial fermentation of fibre energy in the large intestine. The effective ME of digested fiber may be taken as around 0.5 of the DE value. ME is generally about 0.96 of DE, varying from 0.94 to 0.97; the lower value applying when there is a high rate of deamination and urinary N excretion due to either over-supply of diet protein or the supply or poorly balanced dietary amino acids.

On the other hand, Net energy (NE) system has been researched to apply many animal feed companies and research laboratory for the past 10 years. The reason is that many studies showed the positive results of 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). Moreover, saved energy cost per gain by exact prediction of rate of growth and body composition, decreased environmental pollution source like nitrogen, and decreased protein content in diets was studied as positive effects of NE system.

Various studies have been conducted for 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).

2.2 Amino acid (Lysine) Requirements

Many experiments to evaluate the Lys requirement of the gestating sow have conducted. Srichana(2006) studied the Lys requirement of gilts using N balance and suggested a requirement estimate of at least 15 g/d SID Lys in early to mid-gestation and increasing to 20 g/d in late gestation. Although this study suggested a dramatic increase in Lys estimates compared with the 1998 NRC, several considerations need to be addressed in the interpretation. First, these results were collected in gilts, where one would expect very large Lys demand based on maternal weight gain. Second, although the study emphasized linear increase in N retention, it failed to mention that the response was also quadratic. If the quadratic response were examined, then Lys estimates would be from 11 to 14 g in early gestation and from 15 to 19 g in late gestation. The exact requirement and its application could be arguable data, but the latest data suggest that Lys needs are greater than previously estimated, especially in late gestation.

Using the indicator amino acid oxidation method, Samuel et al. (2012) suggested a requirement of 13.4 and 18.7 g/d SID Lys in early and late gestation of second-parity sows, respectively. For third-parity sows, the Lys requirement decreased to 8.2 and 13.0 g/d in early and late gestation, respectively. Using the indicator amino acid oxidation procedure, the authors concluded that lysine requirements were less than previously estimated in early gestation, but like Srichana (2006), they found a nearly twofold increase from early to late gestation.

Samuel et al. (2012) also suggested implementing a parity-segregated feeding program to cover the large changes in Lys requirements between gilts and second-parity sows compared with older, more mature sows.

Ji et al. (2005) calculated amino acid needs based on quantitative changes in body composition during gestation. Based on maintenance, maternal tissue, and conceptus gain, they determined that gilts required 6.8 and 15.3 g SID Lys before and after day 70, respectively. Again, their data support a more than twofold increase in Lys from early to late gestation.

A unique aspect of increasing Lys concentrations fed in late gestation is its potential to affect pig and litter birth weight. Yang et al. (2008) observed that increasing total dietary Lys from 0.62% to 0.82% in late gestation not only increased BW and backfat thickness of sows going into the farrowing house, but also increased litter birth weight. Heo et al. (2008) observed similar findings. Zhang et al. (2011) conducted a study evaluating total Lys concentrations ranging from 0.46 to 0.74% of the diet. They observed that increasing total lysine to 0.65% (providing 14.3 g total Lys from Day 30 to 80 followed by an increase in feed intake providing 19.5 g/d total Lys from Day 80 to 110) increased sow BW, backfat gain, and pig birth weight.

The wide range of experimental procedures and conditions suggest that gestating gilts need approximately 10 to 13 g of SID Lys in early gestation and up to 17 to 18 g/d in late gestation. In older sows, however, it appears these levels can be decreased.

2.3 Energy and protein effects on sows

2.3.1 Reproductive development and performance of sows

It is not clear yet that the influence of nutrition during the nursery period on succeeding reproductive performance and longevity in replacement gilts. Under-

nutrition at an early age can influence succeeding reproductive development, but the effects may only be clear when early growth has been severely stunted and the animal fails to compensate in later phases (Dyck et al. 1988). In general, there was no effect of nutrition during the growing period on age at puberty when the nutrient supply is adequate for commercially acceptable growth rates (Huges, 1982). Moderate to severe restriction of energy (below 80% of ad lib feed intake) could delay the onset of puberty in gilts (Zimmerman et al. 1960). Almost ad libtum feeding during rearing increases number of ovulations and potential litter size in gilts (den Hartog et al. 1980). Feeding a high energy or feed intake level prior to breeding that were previously restriction-fed increases ovulation rate to the level that would be obtained if the gilt was maintained on a high level of diet intake (Aherne et al.1985). The optimum duration of the high-energy flushing regime appears to be 11-14 days before the expected date of estrus or mating and can increase number of eggs ovulated by 1-2 (Anderson et al. 1972). The increased eggs ovulated sets the maximum potential litter size. After mating, it is advisable to reduce feed or energy level to gestation like amounts because of the increased chance of embryonic mortality due to increased progesterone clearance observed with high feed intake (den Hartog et al. 1980). Moderate protein restriction during the rearing period does not appear to influence age at first estrous in gilts, although severe protein restrictions or the imbalanced intake of essential amino acids have been demonstrated to delay the onset of puberty (den Hartog et al. 1980; Aherne et al.1985; Friend, 1973). Neither the level of dietary protein (Fowler and Robertson 1954) nor the source of protein during pre-puberty has a significant effect on ovulation rate or embryonic mortality. With longer periods of protein deprivation (greater than one cycle) ovulation rate is reduced and some individuals become anestrus (Mcgillivray et al. 1964; Pond et al.1968)

2.3.2 Lifetime productivity and longevity of sows

The ability to keep females productive through several parities is essential to maximizing the efficiency of a breeding program. Higher culling rates result in an increased proportion of gilts in the herd, while gilts generally have smaller litter sizes compared to later parity sows, and thus a decrease in overall herd productivity occurs. Optimum energy (and feed) intake during rearing depends largely on gilt body composition (Long et al.1998). For very lean gilts, a moderate protein, high-energy diet during rearing optimizes lean growth while encouraging accumulation of body fat stores, improving lifetime performance. Several studies indicate that gilts with a high capacity for lean growth are often more difficult to increase body fat stores, but also if gilts achieve a body weight of at least 135 kg and a minimum P2 backfat of 0.5 inches (12 mm), increasing fat level does not substantially improve lifetime retention (Foxcroft et al 2004; Williams et al 2005). However, average lean gilts may need to have energy restricted slightly during development to avoid excessive body conditioning that can lead to increased farrowing difficulty, decreased feed intake and milk production during lactation, and increased locomotive problems later on due to greater body weight.

The goal of restricting energy and protein intake during rearing is to limit mature body size, thereby minimizing feet and leg problems due to increased body weight. Although gilts reared on lower energy diets tend to farrow more litters before being culled (Foxcroft et al 2004; Williams et al 2005), they also tend to reach puberty later.

Gilts can be fed ad libitum until mating or limit fed beginning around 80 – 90 kg bodyweight. In high-lean genotypes, voluntary feed intake is often somewhat reduced in relation to energy requirements compared to medium- and low-lean females, so ad libitum feeding to ensure adequate body fat stores is preferred with high lean genotypes. In medium- or low-lean genotypes, gilts will tend to consume

more energy than is needed to achieve ideal body condition, thus becoming too fat, so limit feeding is advised with those genotypes after selection has occurred. Regardless of the feeding approach used, replacement gilts' feed needs to meet the energy, amino acid, mineral, and vitamin needs for growth and development. The feeding program should be designed to ensure that gilts do not become overly fat or grow too fast, resulting in increased body weight and potential feet and leg problems due to excessive weight.

2.4 Energy in Gestation Diet of Sows

Energy requirements according to NRC (2012) consists six tissue pools for maintenance, growth (maternal protein and fat deposition), fetus, mammary tissue, uterus, and placenta and fluids. Maintenance and maternal growth is the greatest portion of the energy requirement.

Data from Noblet et al. (1985) are useful in estimating the energy requirement for growth of the fetus, placenta and fluids, and mammary tissue. As an example, the energy required for fetal, placental, fluids, and mammary tissue increases from less than 400 kcal/d in mid-gestation to over 1,500 kcal/d in the last week of gestation for sows farrowing 12 to 13 pigs. Young et al. (2004) observed that the energy needed for sows to meet their energy requirements for maintenance, uterine, fetal, and mammary growth (no maternal energy retention) increased from approximately 6.02 Mcal ME/d on day 90 of gestation to 8.27 Mcal on day 110. This increase of 2.25 Mcal is greater than the earlier estimates from Noblet et al. (1985), most likely because of the greater litter size (15 to 17 pigs) in the experiment by Young et al. (2004).

Most studies have indicated that feeding increased energy in late gestation did not influence piglet birth weight but that it increased maternal weight gain and might influence pig survivability (Weldon et al., 1994; van der Peet-Schwering et

al., 2004). Conversely, Coffey et al. (1994) found that increasing energy intake during gestation from 5.9 to 7.4 Mcal of ME/d increased pig birth weight and preweaning weight gain, but the high-energy diet during gestation also resulted in decreased feed intake during lactation. The birth weight response in this experiment may have been a result of control sows being provided only 5.9 Mcal of ME/d. This is below typical energy amounts provided in gestation. Weldon et al. (1994) observed that sows fed ad libitum from day 60 of gestation to farrowing had reduced insulin sensitivity and thus had impaired glucose tolerance. Along these lines, Kemp et al. (1996) observed that sows that were less glucose tolerant had greater postnatal piglet mortality; in that study, poor glucose tolerance was a risk factor for preweaning survival. van der Peet-Schwering et al. (2004) found this effect only with high fat, but not high-starch feeding. Feeding sows fat in late gestation made them less glucose-tolerant and was a risk factor for increased stillbirths; however, Averette Gatlin et al. (2002) observed that supplementing high levels of medium- or long-chain triglycerides had no effect on pig survival but did increase the number of stillborn pigs.

Energy source fed in late gestation can also influence composition of the colostrum and survival of piglets when preweaning mortality is greater than 15%. In most studies, no effect of level of fat or starch feeding in late gestation has been found on piglet mortality after birth (Pettigrew,1981; Azain, 1993). When preweaning mortality is affected, it is normally in herds with high preweaning mortality >20% (Seerley et al., 1974; Pettigrew, 1981). Some studies have shown improvements in preweaning mortality, especially in lightweight pigs, due to feeding longchain tallow (Okai et al., 1977) or medium-chain (Azain, 1993; Jean and Chiang, 1999) triglycerides. Newcomb et al. (1991) suggested that any positive benefits to feeding a long-chain fat (soybean oil in their studies) during late gestation are likely mediated by a change in fatty acid composition of colostrum,

whereas benefits to medium-chain triglycerides are most likely mediated through improved blood glucose maintenance of the piglet when it is subjected to fasting conditions. Other ketogenic agents such as 1,3, butanediol also have been shown to improve piglet survival when fed to sows in late gestation (Rosebrough et al., 1981; Stahly et al., 1986) because they can readily cross the placenta and provide an energy source to spare glucose and increase fetal energy stores at birth. Strategies that improve the energy stores of baby pigs during late gestation such as ketogenic agents like 1,3 butanediol or medium-chain fatty acids have a greater likelihood to improve survivability of piglets after birth than attempts to change composition of the colostrum.

3. Fat in Diets of Swine

3.1 Fat Supplementation in Diets of Gestation Sows

Most studies of sow nutrition during gestation focus on preparation for parturition and lactation. Studies have targeted nutrition during late gestation or lactation, or both periods. Fat supplementation for diets of late gestation sows has shown increased milk yields (Coffey et al., 1982). On the other hands, other studies have shown negative effects on sow performance during lactation. In gilts increased energy intake during late gestation hinder the development of mammary secretory tissue (Weldon et al., 1991), and increased the loss of body condition due to reduced feed intake. According to NRC (2012), fetal energy demands are greatly increased during late gestation, and catabolism of maternal reserves occurs if dietary energy supply is insufficient to meet requirements (Boyd et al., 2002). Revell et al. (1998) reported that increasing the energy intake of sows during the anabolic phase of early-mid gestation is likely to improve the amount of fat available for mobilization during late gestation. It also could be result in enhanced sow performance during lactation period.

The fatty acid composition of sow diet could affect the fatty acid composition of body fat reserves in pigs (Huo et al., 2003). According to some studies about rodent, fat accumulates during early pregnancy (Lopez-Luna et al., 1986), as a result of enhanced insulin sensitivity in enhanced adipose tissue lipoprotein lipase activity (Knopp et al., 1973). That stimulates maternal fat reserves to be laid down during early gestation, being later mobilized during late gestation and lactation period. Therefore, the fatty acid composition of maternal fat reserves would influence milk composition that can affect directly piglet growth and progeny survival (Cieslak et al., 1983; Rooke et al., 2001). Also, King (2000) reported that milk yield is influenced by the number size and vigor of piglets. An enhanced milk fatty acid profile caused by maternal fat supplementation lead to increased piglet vigor; leading to increased milk yield, which acts synergistically to improve piglet growth performance and development, further enhancing milk yield.

3.2 Fat Supplementation in Diets of Lactation Sows

Many studies have reported that addition of fat to the diets of sows during late gestation and lactation improves milk production and fat content of colostrums and milk (Pettigrew, 1981). Some studies also have shown an increased survival of piglets by feeding fat to sows before farrowing (Moser and Lewis, 1980; Pettigrew, 1981), as well as improvement in piglet weight gain (Boyd et al., 1978; Tilton et al., 1999), and a decrease of the body loss of the sows during lactation (Kornblum et al., 1991). The positive effects have been reported in studies where fat levels between 7.5% and 15% were used. In some studies, the supplemental fat to the diets can affect milk fat content. However, other studies reported that the effects on sow and litter performance are more variable. Also, there were no significant improvements of litter performance (Boyd et al., 1982; Kornblum et al., 1991) or body weight and backfat thickness of sows and milk production could be shown by supplementation

of fat to sows diets, whereas the research by Drochner (1989) indicated that addition of animal fat resulted in more body weight loss of lactation sows. Some studies have suggested that starch source is superior to fat as an energy source in lactation diets of sows (Jones et al., 2002). The dietary fat content of diets enhanced to reduce mobilization of body stores.

When feeding high fat diets a reduction of feed intake is often seen in sows. However in a review on high fat diets, Drochner (1989) showed that in older parity sows total ME intake was still increased by about 3-32% (as a mean 12 %). Fat as an energy source also seems to increase milk fat content and in some cases total milk output (Pettigrew,1981; Drocher, 1989). Van den Brand et al. (2000) measured energy and protein balances in primiparous, isocalorically fed sows with diets containing 13.5 % fat as compared to diets with 3.4 % fat at two different feeding levels. At high feeding levels the fat rich diet resulted in an increased milk fat content and a significant higher body fat loss from the sows. Over a 21 day lactation period this means that the fat rich diet results in 3.8 kg more loss of fat reserves as compared to the starch rich diet. At low feed intake levels losses of body condition were similar for both diets. Fat rich diets may be beneficial in a hot climate since heat production of sows is lower when fat is used for milk production instead of carbohydrates. However the milk fat driving effect of the fat rich diet makes it unlikely that fat rich diets will help the sow to prevent loss of body condition even when the energy intake is higher. It is therefore also questionable whether high fat diets are beneficial for prevention of reproductive problems in the primiparous sows.

3.3 Fat Sources and fatty acid of pig diets

Fats and oils are important ingredients in diets owing to their high energy value. However, the fats and oils used in animal diets are extremely diverse in

chemical composition, which influences the digestibility and the energy value (Wiseman & Cole, 1987; Gjern & Andersen, 1991). Variation in fatty acid composition of the fat and the fat digestibility are important determinants of the energy value of fat sources as a dietary ingredient for animals (Wiseman et al., 1990). In experiments with pigs, where fatty acid digestibility was measured at both ileal and faecal level, it was found that microbial hydrogenation occurs to a great extent in the hindgut (Jørgensen & Just, 1988). Thus, the amount of fatty acids being absorbed has to be measured at the ileal level assuming negligible fermentative activity, whereas total fat digestibility can give reliable results at the faecal level (Jørgensen et al., 1992a). Fats of animal origin are normally considered to have a lower digestibility and hence lower energy value than fats of vegetable origin, owing to the higher content of saturated fatty acids in the former (16:0, 18:0), which have lower ileal digestibility than the unsaturated fatty acids 18:1, 18:2 and 18:3 (Jørgensen et al., 1992a, 1993; Øverland et al., 1994). The dietary mineral content does not seem to influence the ileal fatty acid digestibility, whereas increasing dietary protein has a positive influence on the digestibility of some saturated fatty acids, such as 14:0 and 18:0 (Jørgensen et al., 1992a). Although the effect of protein on fatty acid digestibility is marginal, the apparent ileal digestibility of most of the amino acids in a soy bean meal-based diet fed to young pigs increased linearly with increasing addition of dietary rapeseed oil (Li & Sauer, 1994).

The efficiency of utilization of metabolizable energy (ME) from fat is higher than the efficiency of ME from protein, starch or fibre (Just, 1982a; Just et al., 1983; Noblet et al., 1993), mainly because fat or absorbed fatty acids can be deposited in adipose tissue without any biochemical transformations. Dietary fatty acid composition reflects that of sow's milk and subsequently of adipose tissue in weaning piglets (Farnworth et al., 1994). Moreover, the amount and fatty acid

composition of the diet has a significant impact on the carcass fatty acid profile, with a subsequent influence on both palatability and nutritional quality, as well as the processing properties (Madsen et al., 1992; Morgan et al., 1992).

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Chapter III: The Effect of VevoVitall® Supplementation on the Performance, Nutrient Digestibility, Blood Profile and Ammonia Gas Emission in Weaning and Growing Pigs

ABSTRACT: The experiment was conducted to investigate whether VevoVitall® supplementation provided by DSM Nutrition Korea Ltd. was suitable as an antibiotic alternative and would produce a synergistic effect with antibiotics on growth performance, urine pH, blood profile, nutrient digestibility and ammonia emission in weaning and growing pigs. The experiment consisted of 4 treatments: 1) Ncon (negative control), basal diet (corn-soy bean meal based); 2) Pcon (positive control), basal diet + Antibiotic colistin sulfate 0.12%; 3) NeVe, basal diet + VevoVitall® 0.5%; and 4) PoVe, basal diet + Antibiotic colistin sulfate 0.12% + VevoVitall® 0.5%. Treatments were applied during weaning (5 weeks) and growing (6 weeks) periods. Crossbreed pigs (n = 128; [Landrace x Yorkshire] x Duroc) weaned at 24 ± 3d of age were used. During weaning, body weight (BW), average daily gain (ADG) and average daily feed intake (ADFI) were higher in treatment Pcon, NeVe and Pove than in Ncon ($P<0.05$). No difference was observed among NeVe and PoVe with added VevoVitall® supplementation to diet and Pcon. Effects during the weaning phase affected the growth performance in the growing phase. During the growth phase, there was no observed significant difference in ADG; however, Pcon, NeVe, and PoVe tended to show a more positive effect than Ncon. In this experiment, the results did not reveal an interaction or synergistic effect between antibiotic and VevoVitall® supplementation; however, the growth performance of PoVe was greater than that in the other treatments. No significant differences were found in nutrient digestibility, blood urea during the weaning and growing phases. In the case of urine pH trial, urine pH was lower ($p=0.069$) in diets

with added VevoVitall® supplementation (NeVe and PoVe) in the weaning phase. Also, in the growing phase, treatment PoVe (antibiotic with VevoVitall® supplementation) showed nearly significant lower urine pH (P=0.059). The pH in feces tended to be lowered in pig fed diets supplemented with benzoic acid (NeVe and PoVe) than in pig fed diets without benzoic acid by 9.01% and 4.05%, respectively, and ammonia gas emission was also reduced by benzoic acid supplement; however, the difference was not statistically significant. We investigated white blood cell, red blood cell, lymphocyte, total protein and albumin level to determine if there were immune responses detected in blood related to VevoVitall® supplementation. However, no significant differences were observed among all treatments. In conclusion, the results from this experiment suggested that VevoVitall® supplementation of pig diet had a significant influence on the growth performance and a nearly significant effect on urine pH.

Key words: VevoVitall®, Antibiotics, Weaning pig, Growing pig, Growth performance, Nutrient digestibility, pH, Immune

INTRODUCTION

Antibiotics have been used for many decades to improve livestock growth performance and to prevent various diseases in livestock feeding environments. While antibiotics are known to enhance livestock growth, efforts by consumers concerned with antibiotic resistance have put increasing pressure on livestock producers to reduce the use of antibiotics. In addition, reducing or prohibiting the use of antibiotics has become a global issue as, in the European Union (EU), supplementation of antibiotics in animal feed for use as an animal growth promoter (AGP) was completely banned as of January 1, 2006. Therefore, research into antibiotic alternatives has become a popular field of interest. In the EU, where the use of antibiotics as AGPs has been legislated, only 16 feed additives, recognized as antibiotic alternatives, are currently authorized.

Such alternatives can be divided into several groups: acidifiers, probiotics, herbs, nucleotides, vitamins, minerals, non-starch polysaccharides, and enzymes. Among these alternatives, acidifiers have been considered as attractive diet additives for weaning pigs (Kim et al., 2005). Acidifiers are reported to be conservation and protection agents that can protect raw materials against fungi and yeasts. Furthermore, organic acids can improve protein digestibility and have a bactericidal effect.

Lowering dietary pH through the use of weak organic acids has received much attention as alternative to the use of antibiotics. The inclusion of organic acids in the diets of weaning and growing pig has been reported in numerous papers (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Partanen and Mroz, 1999; Risley, 1990). Generally, in those studies, addition of 1–2% of organic acids have been included in pig diets, and improved nutrient digestibility has been reported in several of the experiments (Walsh et al., 2006).

Ammonia emissions from pig production facility can result in environmental pollution (Apsimon and Kruse-Plass, 1990). Urea excreted via pig urine is a major source of such ammonia emission, and such urea is converted into ammonia and carbon dioxide by fecal urease. According to previous articles (Muck and Steenhuis, 1981; Stevens et al., 1989; Sommer and Husted, 1995), the most important factors affecting this conversion process are the urinary urea concentration, urine pH, and temperature of the urine–feces slurry (Lee, 2006)

One approach to reducing ammonia emission is through the addition of benzoic acid to the pig diets (Brok et al., 1999; Canh et al., 1998a; Hansen et al., 2007). Benzoic acid absorbed in the small intestine reacts with glycine, an amino acid that is usually in excess, and produces hippuric acid. Urine pH is lowered by the hippuric acid (pKa 3.8) (Kluge et al., 2005; Torralardona et al., 2007), and the decreased urine pH level reduces urease activity. Consequently, production of ammonia from the urea in the manure may be reduced.

The aim of this experiment was to investigate whether benzoic acid can be used as an antibiotic alternative or it has synergistic effects with colistin sulfate.

MATERIALS AND METHODS

Animal and Experimental Design

This experiment was conducted at the Seoul National University Swine Research Farm in Suwon, Korea for 11 weeks (a 5 week weaning period and a 6 week growing period). Crossbred pigs (n=128; [Landrace × Yorkshire] × Duroc), weaned at an age of 24 ± 3 d were allotted on the basis of weight and sex, then immediately placed in nursery pens (n=4 per pen) by randomized complete block design (RCBD). The experiment consisted of 4 treatments: 1) Ncon (negative control), basal diet(corn-soybean meal based); 2) Pcon (positive control), basal diet + Colistin sulfate 0.12%; 3) NeVe; basal diet + VevoVitall® 0.5%; and 4) PoVe, basal diet + Colistin sulfate 0.12% + VevoVitall® 0.5%. There were 8 replications per treatment.

Experimental Diet

Diets provided during the 3 phases (weaning phases I and II, and growing phase) were formulated using corn-soybean meal mixtures. Weaning phase I diet contained a lysine (total) level of 1.35% and was fed from day 0 to 14 of weaning, the phase II diet contained 1.15% lys (total) and was fed from day 15 to 35 of weaning. The growing phase diet contained 0.95% lys (total) on an as fed basis and was provided from day 36 to the end of the experiment. The basal diets contained approximately metabolizable energy (ME) of 3,279 and 3,265kcal/kg in the weaning and growing periods, respectively. The basal diet met or exceeded all NRC (1998) nutrient requirement estimates. The formula and chemical compositions of the basal diets in all phases are presented in tables 1–3.

Housing and Management

All pig were housed in half-slotted concrete floor pens (0.90 m × 2.15 m^2 for four pigs) during the weaning phases, and moved into concrete-floored half-plastic woven slurry pens (1.26 m × 2.55 m^2 for four pigs) during the growing phase. Feed and water were available for *ad libitum* consumption throughout the experimental period. The room temperature was initially set at 30°C and adjusted downward as needed to meet the perceived comfort zone of the pigs. Individual pig body weights (BW) were recorded initially and at the end of each phase (i.e., at days 14, 35, 56, and 77). At the conclusion of the experiment, the data were summarized, and the average daily gain (ADG), average daily feed intake (ADFI) and the feed efficiency were calculated for each phase and for the entire experimental period.

Metabolic Trials

Metabolic trials were conducted twice: once during the weaning period and once in the growing period. In the weaning period trial, 16 barrow pig (at age 30d) with average initial BW of 9.48 ± 0.49 kg, were allocated to the 4 treatments with 4 replicates and placed, using a complete randomized design (CRD), in metabolic crates fitted with urine and feces separators. In the growing period trial, 12 barrow pigs (average initial BW 38.90 ± 2.29 kg) were used. All pig were acclimated to the metabolism crates for 1–2 d before undergoing a 7d of adaptation to their respective dietary treatments. During the dietary adaptation period, feed intake levels were equalized within replicates to that of the pig consuming the least amount of feed. All pigs were provided their daily diet at 07:00 and 19:00. After 7 d of adaptation, all pigs were subjected to a 5d collection period. The total amount of feed consumed and excreta produced were recorded daily during each metabolic trial. Collected excreta from each pig were stored at −20°C. After collection, the excreta were dried in a forced air drying oven at 60°C for 72 h and ground to a 1

mm particle size in a Wiley mill for chemical composition analysis.

Urine pH

Urine pH values were measured in both weaning and growing phases. In the weaning period, 16 barrow pigs (at 42 day) were allocated, using CRD, to the 4 treatments with 4 replicates and placed in metabolic creates fitted with urine and feces separators. All pigs were provided their daily allotment of feed at 07:00 and 19:00. Urine was collected individually just after feeding using a tube with a funnel. Collections were made for 4 d after a 7-d adaptation period. The pH of the urine samples was immediately measured by pH meter. In the growing period, 12 barrow pigs were similarly allocated, fed, and sampled prior to measuring urine pH.

Ammonia gas emission and pH in Feces

Twelve growing barrow pigs were allocated by CRD to 4 treatments with 3 replicates and placed in metabolism creates. The experimental diet was provided at a rate of 800 g per pig per day, with feeding twice per day during a 7-d adaptation period and for a subsequent 4-d feces collection period. The pH in the feces was directly analyzed by pH meter (Model IQ150, IQ Scientific Instrument, SanDiego, CA, USA). Subsequent to the pH measurement, the individually collected feces samples were mixed with 50 ml of water in flask and the flask placed in a drying oven at 60°C for 48 h with ammonia gas emission measured by gas detector (MDA Scientific Chemkey; Honeywell, Sunrise, FL, USA).

Blood Profile

Blood samples were collected from individual pig (selected by body weight) at the end of each phase for determination of WBC (white blood cell), RBC (red blood cell), lymphocyte, total protein, and albumin levels. Approximately 5 ml

blood was collected by K3EDTA vacuum tube (Becton Dickinson Vacumtainer Systems, Franklin Lakes, NJ, USA) from the jugular vein. The serum was centrifuged immediately and the aforementioned determinations made. In addition, blood samples were collected in the same manner from the jugular vein of the same pig at the end of each phase of the experiment for blood urea nitrogen (BUN) analysis. Collected blood samples were immediately centrifuged for 15 min at 3,000 r/min and 4°C. The serum was carefully removed, placed in plastic vials, and stored at −20°C until BUN analysis. Total BUN concentrations were analyzed using a blood analyzer (Ciba-Corning model, Express Plus, Ciba Corning Diagnostics, Cambridge, MA, USA).

Statistical and Chemical Analysis

Analyses of experimental diets and excreta were conducted according to the methods of the AOAC (1995). Performance (ADG, ADFI, G:F ratio) data were analyzed uisng a randomized complete block (RCB) design using the General Linear Model (GLM) procedure of SAS (2004). The individual pen was the experimental unit for analysis of performance data. For nutrient digestibility, the analysis was performed using the SAS GLM procedure with the individual pig as the experimental unit. Comparison of means was performed according to the least significant difference (LSD) multiple range tests of SAS.

RESULTS AND DISCUSSION

Growth performance

The effects of VevoVitall® supplementation on growth performance during weaning (5 weeks) and growing (6 weeks) periods in pig are presented in Table 4. During weaning phase I (0-2 weeks), Pcon and Pove treatment groups showed significant differences in BW, ADG, and G:F ratio compared to Ncon ($P<0.05$). Pig fed with an antibiotic diet (Pcon, PoVe) showed the highest growth performance during first 2 weeks, followed by pig fed a diet supplemented with VevoVitall® only, while Ncon pig had the lowest growth rate throughout the weaning phase. Some studies (Held and Mendl, 2001; Jensen, 2002; King and Pluske, 2003) reported that the important factors causing stress during pig weaning are separation from the sow, transport, changing nutrition, different pathogenic pressures from the new environment, as well as increased aggression as new dominance hierarchies are established following commingling of previously unfamiliar piglets. In this experiment, the growth performance of the pig group of basal diet without antibiotic or VevoVitall® supplementation was not improved and the group also showed a growth lag during the first two post-weaning weeks. This post-weaning lag was not evident in the other experimental groups, suggesting that the lag could be alleviated by antibiotic or VevoVitall® supplements. This tendency, i.e., that treatments Pcon, Neve, and Pove showed significantly better results on growth performance such as BW, ADG and ADFI, than the Ncon treatment continued during the entire weaning phase (5 weeks) ($P<0.05$).

The effects of treatments during the weaning period affected the growth performance of the subsequent growing period (5th-11th weeks). In treatment groups Pcon, NeVe and PoVe, ADG were increased by 6.78%, 5.65% and 8.62%, respectively, compared with treatment Ncon. In addition, the tendency that pig that

received antibiotic with VevoVitall® supplementation has the highest numerical value of ADG as observed in the weaning period, was also present in the growth period.

The two experiment groups Pcon and PoVe that received antibiotic supplementation showed more ADFI compared with Ncon ($P<0.05$) during growing period. There was a tendency to higher ADFI in the group with VevoVitall® added to the diet without antibiotics than in the Ncon group, but the difference was not significant. In contrast, with regard to feed efficiency, treatment Ncon showed the highest feed efficiency compared to treatments Pcon and Pove; this is related to the gap in total feed intake during the growing period (6th-11th week) ($P<0.05$). Overall, no difference in ADFI was detected between NeVe and the other treatments.

Over the entire experimental period, antibiotic, VevoVitall®, and antibiotic with VevoVitall® supplementations showed tendencies toward increasing body weight, average daily gain, and average feed intake in treated pig. The effect of an antibiotic as a growth promoter has been reported in previous study (Hay, 1977). In addition, acidifier supplementation has produced positive effects on growth performance according to other study (Partanen and Mroz, 1999). Kluge et al. (2005) reported that supplementation of the diet with benzoic acid increased feed intake and body weight gain and improved feed conversion ratio. This is in agreement with the results in our experiment. Pluske et al. (2003) reported that weight gain in piglets immediately after weaning could influence their growth until the end of the fattening period. Thus, the gap in growth performance in the early weaning phase of our study may have affected growth performance throughout the remainder of the experimental period.

The pig group fed the diet with both antibiotic and VevoVitall® supplementation showed higher numerical levels in all growth performance

indicators (BW, ADG, ADFI) than those in other treatments; however, the differences were not statistically significant. According to some previous studies (Petersen & Oslage, 1982; Edmonds et al., 1985; Radecki et al., 1988; Eidelsburger et al., 1992), organic acids can enhance the effect of antibiotics by improving their absorption. In this experiment, there was no indication of a distinct interaction or synergistic effect between antibiotics and VevoVitall®; however, the possibility of such interactions or synergistic effects between the two supplements was suggested by the observed growth performance tendencies.

Nutrient digestibility

The effect of VevoVitall® supplementation on nutrient digestibility and nitrogen (N) retention are presented in Tables 5 and 6. There were no significant differences in digestibility of dry matter, crude protein, crude fat, ash, or crude fiber among the treatments. According to Kluge et al., (2005), piglets fed diets supplemented with benzoic acid at rates of 5 or 10 g/kg retained 5% and 6% more N, respectively, than control piglets. In this experiment, there were no significant differences in N retention or digestibility of other nutrients, even though the G:F ratio could be increased by VevoVitall® or antibiotic treatment during the weaning and growing period.

Blood urea nitrogen

Figure 1 presents the BUN concentration assay results. In general, BUN is an indicator of maximal amino acid utilization, and it has been reported that BUN is related directly to protein intake and related inversely to protein quality and retained dietary N in the body (Eggum, 1970). BUN concentration (mg/dL), along with estimates of N digestibility (g/g) and N retention (g/d) can be used to predict fecal N (g/d), N intake (g/d), and N utilization efficiency. In this experiment, BUN

concentrations ranged between 11.70 and 17.7 mg/dL, with no significant differences detected in BUN levels among treatments. Kil et al., (2006) has also reported that acidifier supplementation did not alter BUN levels in pig.

pH of urine

The effect of VevoVitall® supplementation on urine pH is presented in Table 7 and Figure 2. The pH of urine was measured twice: during the weaning period and the growing period. In the weaning period trial, benzoic acid supplemented groups showed a trend to a lower urine pH than the values in treatments Ncon and Pcon (P=0.069). Also, a nearly significantly lower urine pH was observed in treatment PoVe in the growing period trial (P=0.059). Peet-schwering (1998) reported that urine pH was influenced by the percentage of benzoic acid in diet; the higher the benzoic acid percentage (0%, 1% and 2%) in the diet, the lower the urine pH (7.52, 6.45 and 5.59, respectively). Also, Brok et al.(1999) reported that the average urine pH values of pig fed on control and acidified starter feeds (a mixture of organic acids, but mainly benzoic acid) were 7.50 and 5.69, respectively, and in pig fed on growing and finishing feeds the urine pH values were 7.48 and 5.02, respectively. Additionally, Brok et al. (1999) observed lower ammonia emission after adding a combination of different organic acids, mainly benzoic acid, at a concentration of 1% to starter feed and 2% to the growing-finishing feed. Similar findings, showing that the addition of 3% benzoic acid to pig feed reduced ammonia concentration and ammonia emission by 55% and 57%, respectively have been reported (Hansen et al., 2007). The reduction in ammonia emission by adding benzoic acid to pig feed may be related to a decrease in urine pH (Brok et al., 1999; Cahn et al., 1998a). In this research, nearly significant effects of addition of benzoic acid to the diet on urine pH were observed in weaning periods. Based on these results, when benzoic acid is supplemented in

pig diet, ammonia gas emission could be reduced through a tendency toward a lowering of urine pH.

pH and ammonia gas emission in feces

The pH and ammonia gas emission levels in feces were not significantly different (Table 8). In this experiment, the pH of feces tended to be lower in the NeVe and PoVe treatments supplemented with benzoic acid than in the treatments without benzoic acid by 9.01% and 4.05%, respectively. Ammonia gas emission was also reduced by benzoic acid supplement; however, the difference was not statistically significant. Based on these results, although VevoVitall® may decrease the pH of feces and reduce ammonia gas emission from feces, the decreases are not significant.

Blood profiles

The effects of VevoVitall® supplementation on blood profiles are presented in Table 10. Blood profiles are good indicators of health and disease conditions, therefore, blood samples were analyzed to confirm whether the dietary treatments had any effects on the blood profile of the pigs. Most of the haematological indices recorded in this experiment were within the normal ranges reported by Siegmund and Fraser (1982.) There were no treatment differences in RBC and WBC counts. Furthermore, lymphocyte counts were not affected by the addition of antibiotic or benzoic acid. In Pcon and PoVe treatments, lymphocyte counts tended to increase between day 14 and day 77; however, the difference was not significant. In addition, the lymphocyte counts in treatment Ncon decreased, but not significantly. Furthermore, there were no significant differences in total protein levels and albumin levels among treatments.

IMPLICATION

According to the nutrient digestibility, blood characteristic, and BUN results, there were no significant difference among treatments in this experiment. However, treatments supplemented with VevoVitall® showed better growth performance and marginally lower urine pH than the Ncon treatment group. Such results were similar to the results from the antibiotic supplemented treatment group. Consequently, it appears that feed supplemented with benzoic acid is a possible alternative to feed supplemented with antibiotics. Even though the reduction effects may not be significant, the supplements may contribute to reducing ammonia emission by producing a decrease in urine pH.

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Table 1. Formula and chemical composition of the experimental diet in weaning phase I (0 to 2 weeks of age after weaning)

Ingredients	Ncon	Pcon	A	B
Corn	33.40	33.28	32.90	32.78
SBM, 44% CP	23.02	23.02	23.02	23.02
Pepsoygen	15.41	15.41	15.41	15.41
Whey powder	5.05	5.05	5.05	5.05
Dried skim milk	3.09	3.09	3.09	3.09
Lactose	16.60	16.60	16.60	16.60
Soy oil	0.65	0.65	0.65	0.65
MCP	1.12	1.12	1.12	1.12
Limestone	1.05	1.05	1.05	1.05
DL-Met	0.09	0.09	0.09	0.09
Vit.Mix[a]	0.12	0.12	0.12	0.12
Min.Mix[b]	0.10	0.10	0.10	0.10
Salt	0.20	0.20	0.20	0.20
Choline-Cl, 25%	0.10	0.10	0.10	0.10
Antibiotics[c]	0	0.12	0	0.12
Benzoic acid	0	0	0.50	0.50
Total	100	100	100	100
Chemical Composition [d]				
ME, kcal/kg	3285.46	3281.50	3268.96	3265.00
CP, g/kg	23.05	23.04	23.01	23.00
Lysine, g/kg	1.4	1.4	1.4	1.4
Methionine,g/kg	0.44	0.44	0.44	0.44
Ca, g/kg	0.8	0.8	0.8	0.8
Total P, g/kg	0.66	0.66	0.65	0.65

[a] Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3, 1,600 IU; vitamin E, 32 IU; vitamin K, 2.40 mg; vitamin B_2, 3.20 mg; vitamin B_{12}, 12 g; Ca pantothenate, 8 mg; Biotin, 64 g; Niacin, 16 mg.

[b] Provided the following per kilogram of diet: Fe, 127.3mg; Mn, 24.8 mg; Zn, 84.7 mg; Cu, 54.1 mg; Se 0.1 mg; Co, 0.3 mg; I, 0.3 mg.

[c] Antibiotics: Colistin sulfate 10 g/kg.

[d] Calculated value.

Table 2. Formula and chemical composition of the experimental diet in weaning phase II (3th to 5th weeks of age after weaning)

Ingredients	Ncon	Pcon	A	B
Corn	46.63	46.51	46.13	46.01
SBM, 44% CP	23.67	23.67	23.67	23.67
Pepsoygen®	11.14	11.14	11.14	11.14
Whey powder	5.11	5.11	5.11	5.11
Lactose	9.00	9.00	9.00	9.00
Soy oil	1.69	1.69	1.69	1.69
MCP	1.13	1.13	1.13	1.13
Limestone	1.05	1.05	1.05	1.05
DL-Met	0.06	0.06	0.06	0.06
Vit.Mix[a]	0.12	0.12	0.12	0.12
Min.Mix[b]	0.10	0.10	0.10	0.10
Salt	0.20	0.20	0.20	0.20
Choline-Cl, 25%	0.10	0.10	0.10	0.10
Antibiotics[c]	0	0.12	0	0.12
Benzoic acid	0	0	0.50	0.50
Total	100	100	100	100
Chemical Composition [d]				
ME, kcal/kg	3285.46	3281.50	3268.96	3265.00
CP, g/kg	21.05	21.04	21.01	21.00
Lysine, g/kg	1.21	1.21	1.21	1.21
Methionine,g/kg	0.37	0.37	0.37	0.37
Ca, g/kg	0.75	0.75	0.75	0.75
Total P, g/kg	0.63	0.63	0.63	0.63

[a] Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3, 1,600 IU; vitamin E, 32 IU; vitamin K, 2.40 mg; vitamin B_2, 3.20 mg; vitamin B_{12}, 12 g; Ca pantothenate, 8 mg; Biotin, 64 g; Niacin, 16 mg.

[b] Provided the following per kilogram of diet: Fe, 127.3mg; Mn, 24.8 mg; Zn, 84.7 mg; Cu, 54.1 mg; Se 0.1 mg; Co, 0.3 mg; I, 0.3 mg.

[c] Antibiotics: Colistin sulfate 10 g/kg.

[d] Calculated value.

Table 3. Formula and chemical composition of the experimental diet in growing phase (6th to 11th weeks)

Ingredients	Ncon	Pcon	A	B
Corn	67.93	67.83	67.43	67.33
SBM, 46% CP	28.78	28.78	28.78	28.78
Tallow	1.31	1.31	1.31	1.31
L-Lysine HCl	0.01	0.01	0.01	0.01
Limestone	0.46	0.46	0.46	0.46
TCP	1.01	1.01	1.01	1.01
Vit.Mix[a]	0.10	0.10	0.10	0.10
Min.Mix[b]	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Antibiotics[c]	0	0.10	0	0.10
Benzoic acid	0	0	0.5	0.50
Total	100	100	100	100
Chemical Composition[d]				
ME, kcal/kg	3285.19	3282.17	3268.53	3265.43
CP, g/kg	18.05	18.04	18.01	18.00
Lysine, g/kg	0.95	0.95	0.95	0.95
Methionine,g/kg	0.30	0.30	0.30	0.30
Ca, g/kg	0.60	0.60	0.60	0.60
Total P, g/kg	0.50	0.50	0.50	0.50

[a] Provided the following per kilogram of diet: vitamin A, 8,000.00 IU; vitamin D_3, 31,6000.00 IU; vitamin E, 17.40 IU; vitamin K, 32.40 mg; vitamin B_2, 3.20 mg; vitamin B_{12}, 24.00 ug; Ca pantothenate, 8.00 mg; Biotin, 0.10 mg; Niacin, 16.00 mg; Ethoxyquin, 6,612.00mg.

[b] Provided the following per kilogram of diet: Fe, 95.95 mg; Mn, 85.46 mg; Zn, 90.55 mg; Cu, 24.26 mg; Se 38.35 mg; Co, 1.29 mg; Ca, 2.08 mg; I, 13.20 mg.

[c] Antibiotics: Avilamycin 20 g/kg.

[d] Calculated value.

Table 4. Effect of benzoic acid on the growth performance in weaning and growing pigs

Item	Ncon[1)]	Pcon	A	B	SEM[3)]
Body weight[2)] (kg)					
Initial	7.26	7.27	7.26	7.26	0.15
2nd wk	8.58[b]	9.73[a]	9.56[ab]	9.97[a]	0.19
5th wk	17.68[b]	19.42[a]	19.51[a]	20.19[a]	0.31
8th wk	30.27[b]	33.10[a]	32.98[ab]	34.79[a]	0.53
11th wk	47.41	51.06	50.88	52.69	0.67
Average daily gain[2)] (g)					
0-2 week	94[b]	176[a]	164[ab]	194[a]	15.02
2-5 week	433	464	472	488	9.55
0-5 week	297[b]	349[a]	349[a]	370[a]	8.83
5-8 week	601	653	640	695	12.95
8-11 week	816	860	856	844	14.38
5-11 week	708	756	748	769	11.14
0-11 week	522	571	566	588	8.8
Average daily feed intake[2)] (g)					
0-2 week	211	267	268	280	9.49
2-5 week	670	740	741	768	13.43
0-5 week	475[b]	551[a]	553[a]	574[a]	10.97
5-8 week	1320[b]	1479[a]	1471[ab]	1589[a]	30.11
8-11 week	1834	2055	1996	2116	34.92
5-11 week	1577[b]	1767[a]	1733[ab]	1852[a]	30.31
0-11 week	1077[b]	1214[a]	1197[a]	1271[a]	20.28
Gain : Feed ratio[2)]					
0-2 week	0.394[b]	0.657[a]	0.586[a]	0.653[a]	0.043
2-5 week	0.648	0.615	0.628	0.628	0.008
0-5 week	0.620	0.628	0.630	0.646	0.005
5-8 week	0.451	0.440	0.436	0.439	0.003
8-11 week	0.449[a]	0.416[ab]	0.427[ab]	0.403[b]	0.006

5-11 week	0.449[a]	0.426[b]	0.431[ab]	0.418[b]	0.003
0-11 week	0.484	0.468	0.473	0.465	0.003

[1)] Ncon (basal diet), Pcon(Ncon + antibiotic), A(Ncon + benzoic acid 0.5%), B (Pcon + benzoic acid 0.5%)

[2)] Values are means for eight pens of four pigs per pen.

[3)] Standard error of mean

[a,b] Means with different superscripts in the same row significantly differ (P<0.05)

Table 5. Effect of benzoic acid on nutrient digestibility and nitrogen retention in weaning pigs at phase II [1)]

Item	Ncon[2)]	Pcon	A	B	SE[3)]
Nutrient digestibility (%)					
Dry matter	92.70	91.36	92.89	93.45	0.31
Crude protein	89.68	88.35	90.07	90.31	0.41
Crude fat	85.27	84.47	86.17	87.85	0.55
Crude ash	70.05	65.07	71.43	70.45	1.24
Nitrogen retention (g/day)					
N intake	6.12	6.12	6.12	6.12	-
Fecal N	0.63	0.71	0.61	0.59	0.02
Urinary N	2.07	2.58	2.90	2.30	0.15
N retention[4)]	3.42	2.83	2.61	3.23	0.15

[1)] 16 piglets were used from an average initial body weight of 9.48 ± 0.49 kg

[2)] Ncon (basal diet), Pcon(Ncon + antibiotic), A(Ncon + benzoic acid 0.5%), B (Pcon + benzoic acid 0.5%)

[3)] Pooled standard error.

[4)] N retention = N intake (g) - Fecal N (g) - Urinary N (g).

Table 6. Effect of benzoic acid on nutrient digestibility and nitrogen retention in growing pigs [1)]

Item	Ncon[2)]	Pcon	A	B	SE[3)]
Nutrient digestibility (%)					
Dry matter	91.56	90.62	91.13	92.53	0.38
Crude protein	90.61	90.63	89.59	91.38	0.52
Crude fat	70.19	70.76	73.56	75.13	1.70
Crude ash	68.42	67.73	70.65	74.53	1.58
Nitrogen retention (g/day)					
N intake	26.79	26.79	26.79	26.79	-
Fecal N	2.52	2.51	2.79	2.31	0.13
Urinary N	9.82	10.09	14.26	10.42	0.90
N retention[4)]	14.45	14.19	9.75	14.07	0.90

[1)] 12 pigs were used from an average initial body weight of 38.90 ± 2.29 kg

[2)] Ncon (basal diet), Pcon(Ncon + antibiotic), A(Ncon + benzoic acid 0.5%), B (Pcon + benzoic acid 0.5%)

[3)] Pooled standard error.

[4)] N retention = N intake (g) - Fecal N (g) - Urinary N (g).

Table 7. Effect of benzoic acid on pH of urine in weaning and growing phase

Item (/phase)	Ncon [3)]	Pcon	A	B	SE [4)]
Urine pH					
Weaning phase [1)]	7.74^{a}	7.53^{ab}	7.44^{b}	7.47^{b}	0.06
Growing phase [2)]	6.97^{A}	6.99^{A}	7.04^{A}	6.56^{B}	0.07

[1)] 16 pigs were used from an average body weight of 9.82 ± 0.51 kg .

[2)] 12 pigs were used from an average initial body weight of 42.68 ± 2.11 kg.

[3)] Ncon(basal diet), Pcon(Ncon + antibiotic), A(Ncon + benzoic acid 0.5%), B (Pcon + benzoic acid 0.5%)

[4)] Pooled standard error.

[a,b] Values within same rows with different superscript are significantly different (p=0.069).

[A,B] Values within same rows with different superscript are significantly different (p=0.059).

Table 8. Effect of benzoic acid on pH of feces in weaning and growing phase.

Item	Ncon [2)]	Pcon	A	B	SE [3)]
NH_3 (ppm)	9.18	10.69	7.55	8.61	0.97
pH of feces	5.23	5.30	4.83	5.06	0.11

[1)] 12 pigs were used from an average initial body weight of 42.68 ± 2.11 kg.

[2)] Ncon(basal diet), Pcon(Ncon + antibiotic), A(Ncon + benzoic acid 0.5%), B (Pcon + benzoic acid 0.5%)

[3)] Pooled standard error.

Table 9. Effect of benzoic acid on blood profiles in weaning and growing pigs [1)]

Item	Treatment				SEM[3)]
	Ncon[2)]	Pcon	A	B	
White blood cell ($x10^3/mm^3$)					
Day 14	23.60	24.40	29.80	18.23	2.36
Day 77	30.00	17.97	25.53	20.57	1.65
Difference	6.40	-6.47	-4.30	2.33	-
Red blood cell ($x10^6/mm^3$)					
Day 14	5.93	5.13	6.07	4.11	0.33
Day 77	7.10	7.08	7.08	7.44	0.09
Difference	1.17	1.95	1.01	3.33	-
Lymphocyte (%)					
Day 14	54.00	31.00	49.00	44.33	3.96
Day 77	46.67	68.67	52.00	73.00	4.27
Difference	-7.33	37.67	3.00	28.67	-
Total protein (g/dL)					
Day 14	3.80	3.70	3.83	3.90	0.14
Day 77	5.80	5.13	6.00	5.50	0.19
Difference	2.00	1.43	2.17	1.60	-
Albumin (g/dL)					
Day 14	2.47	2.03	2.50	2.57	0.11
Day 77	3.40	2.93	3.43	3.50	0.12
Difference	0.93	0.90	0.93	0.93	-

[1)] Vaules are means for three pens per treatment.

[2)] Ncon(basal diet), Pcon(Ncon + antibiotic), A(Ncon + benzoic acid 0.5%), B (Pcon + benzoic acid 0.5%).

[3)] Standard error of mean.

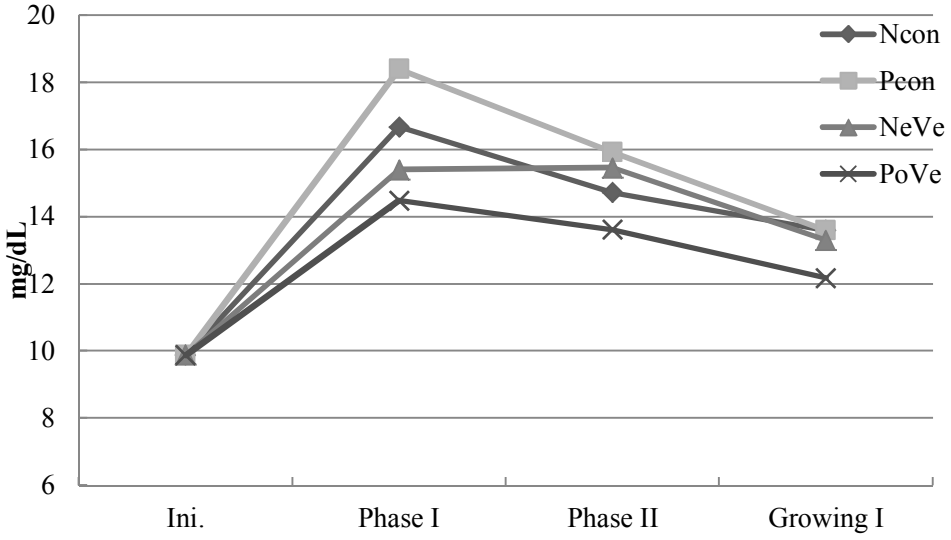

Figure 1. Effect of benzoic acid on blood urea nitrogen in weaning and growing pigs

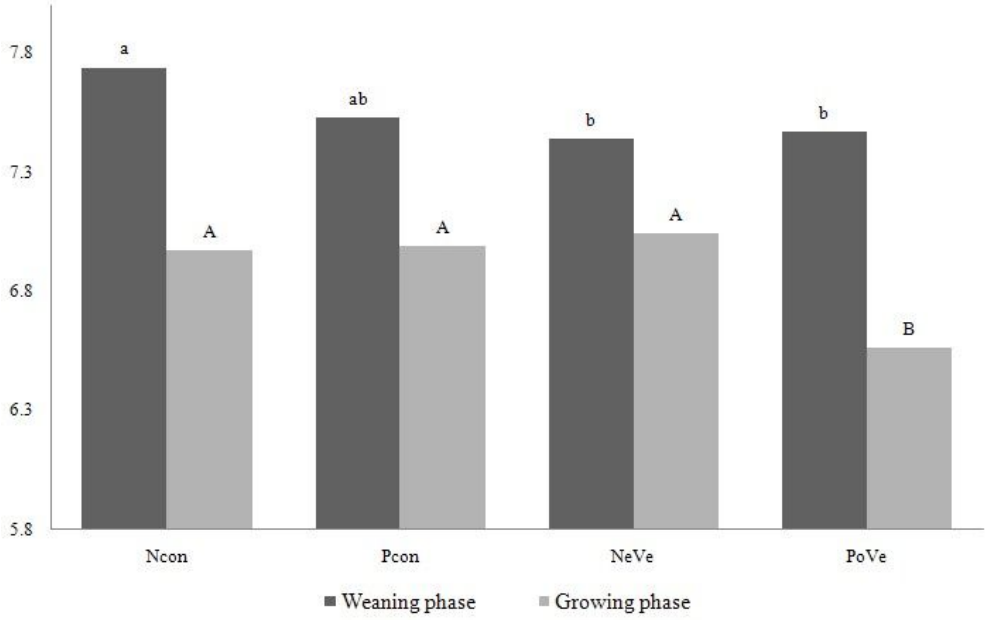

Figure 2. Effect of benzoic acid on pH of urine in weaning and growing pigs

[a,b] Values within same rows with different superscript are significantly different (p=0.069).

[A,B] Values within same rows with different superscript are significantly different (p=0.059).

Chapter IV: Influence of different energy and dietary lysine levels in gestation diets on reproductive performance, blood composition and growth of gilts

ABSTRACT: The aim of this experiment was to determine the influence of different energy and lysine levels of gestating gilts on physiological parameters and reproductive performance of primiparous sows. A total of 30 F1 primiparous sows (Yorkshire x Landrace) were allotted to a 2 x 2 factorial arrangement of treatments in a completely randomized design with 7 replicates. Factors evaluated were energy levels (approx. 3,050 ME kcal/kg or 3,140 ME kcal/kg) and dietary lysine levels (approx. 0.64% or 0.74%). The each experimental diet was provided to gilts of 2.1 kg/d during gestation period. Lysine intake of each treatment gestating gilts was 13.4, 15.5, 13.8 and 15.9 g of total lysine/day, respectively. There were no effects of treatment on the body weight changes from breeding day to 90 d of gestation period ; however, body weight changes during whole gestation period (0~110d) was affected by dietary energy and lysine levels. The gilts fed high energy (3,140 kcal ME/kg) and high dietary lysine level (0.76%) in diet showed higher body weight gain (increasing 65.75 kg) than other gilts ($p<0.05$). No effects of factors (energy level or dietary lysine level) were observed on body weight or its changes during whole gestation period of gilts. Backfat thickness of primiparous for gestation period was not affected by energy lever or level of dietary lysine ($P>0.10$). Higher lysine level (approximately 0.74%) in gestation diet did not show more backfat gain of gilts than low lysine level (approximately 0.64%). The number of piglet per litters and litter birth weight were numerically higher when gilts were fed low energy level (6,405 kcal ME/d) and 0.64 % lysine level diet during gestation period even though it didn't show significant difference among treatments. Also, higher

energy (6,594 kcal ME/d) or higher lysine (15.5g lysine/d) in gestation diet did not affect to reproductive performance of gilts for gestation period. The level of plasma glucose and insulin did not change with high level of energy intake or lysine intake during gestation period. The concentration of blood urea nitrogen at 35 d, 70 d and 90 d in gestation period numerically increased as the dietary lysine level increased ($p<0.05$). Consequently, these results demonstrated that the energy level and dietary lysine level more than around 6,400 kcal of ME/day and 13.4 g/day, respectively, meet the requirements without any negative effects on growth performance and reproductive performance on gilts.

Key words: Energy Level, Dietary Lysine Level, Gilt, Physiological Parameter

INTRODUCTION

Modern prolific sows have advanced and changed to require better nutrition and feeding strategies because of larger body size and the substantial decline in body fat. Further improvement is a certainty, as the threshold of 30 PSY becomes a realistic goal for commercial swine farms. Due to limited data in pregnant sows, most of these requirements were derived from data from growing pigs. This limited study could explains the wide range of recommendations found in the literature for the crude lysine requirement of pregnant sows from 7.7 to 12.4 g/d (ARC,1981; INRA, 1989, NRC, 2012).

Many studies have been conducted to establish energy requirement for gilt and 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 day 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.

Thus our objectives were to examine the influence of different levels of energy and dietary lysine in diet during gestation period for high producing primiparous sows and their reproductive performance

MATERIALS AND METHODS

Animal Management and Experimental Diets

A total of 30 crossbred gilts (Yorkshire x Landrace; Darby Genetics Inc. Anseong, South Korea) with an average bodyweight of 95.0 ± 10.0 kg were housed in environment controlled pen (2.5 x 3.5 m^2) with 3 animals per pen at 180 d of their age for 40 ± 10 days. Animals were given free access to feed and water through feeder and water cup nipple. On d 220 ± 10 of age, gilts were moved into an individual gestation stall (2.4 x 0.64 m^2) and fed diet twice daily. Estrus detection was carried out twice at 9 am and 5 pm by backpressure testing and mature boar contacting. The onset of estrus was determined as the time of occurrence of a standing reflex in the presence of a boar. To minimize any effect of boar to experimental design, gilts (approximately 240 day of age and 140 kg of bodyweight) were artificially inseminated (Darby AI center, Choongju, Korea) with fresh semen. All inseminations were carried out by the same time, same trained person, at 12 h and 24 h after onset of estrus. All gilts were fed individually twice daily at 08:30 and 16:30 h. Daily feed intake of experimental gilts was adjusted to 2.1kg until farrowing. Daily feed allowance was calculated for every gilts based on diet as experimental design. Gilts had *ad libitum* access to water through nipple drinker. Pregnant sows were moved to farrowing house after washing with an antiseptic solution on 110 day. Sows prepared to give birth to their progeny into environmentally controlled farrowing rooms and placed in individual farrowing crate (2.50 x 1.80 m^2).

This experiment evaluated the influences of different energy and dietary lysine levels in gestation diets on reproductive performance, blood composition and growth of gilts. The influences of energy and lysine were evaluated as a 2 x 2 factorial arrangement of treatments in a completely randomized design (CRD) with 7 replicates. Factors evaluated were energy levels (approx. 3,050 ME kcal/kg or

3,130 ME kcal/kg) and dietary lysine values (approx. 0.65% or 0.75%). All gilts were allotted to 4 treatments based on body weight and backfat thickness by completely randomized design (CRD). Diets were corn-wheat bran based, with 7-12 % soybean meal and 3 % coconut meal (Table 1.) Tallow in diets was added at the expense of corn starch to increase energy levels. The first and third treatment dietary lysine levels were 0.64% of diets and the second and fourth treatment dietary lysine levels were 0.74%. These diets were designed to meet or exceed the nutrient requirements for gestating gilts set forth by the NRC (1998). Calculated values of crude fat of each treatment were 6.34%, 6.63%, 8.14% and 8.43, respectively.

Sample collections and analysis

BW and BF thickness of gestation sows were recorded on 0, 15, 35, 70, 90, 110 day and farrowing day. Ultra-sound (Renco Corp., Minneapolis, Lean-meter, USA) was used for measuring BF thickness at the P2 position (mean value from both side of the last rib and 65 mm away from the backbone). Litter weight at 24 h postpartum was recorded. The number of total born, born alive and stillborn were recorded within 24 h postpartum. Blood samples of gilts for analyzing glucose, insulin, protein, blood urea nitrogen and free fatty acid levels at 15, 35, 70, 90 and 110 day of gestation as well as 24 h postpartum were taken from jugular vein. The blood samples were collected into EDTA tubes, and then centrifuged at 3,000 rpm in 4℃ for 15 min (Eppendorf centrifuge 5810R, Hamburg, Germany). Plasma was harvested from all blood samples kept at -20℃ until analysis.

Plasma FFA concentration was determined using NEFA HR. II kits (Wako chemical, Osaka, Japan) and content of plasma glucose was detected by enzymatic kit (Glucose Hehokinase Kit, Bayer, Pennsylvania, USA). Insulin levels were analyzed using ECLIA (Electrochemi iuminescence immumoassay) kit. Total BUN

concentrations were analyzed using Urea nitrogen Reagents (SIEMENS, USA). Also, Protein level in blood was detected by Total protein Reagents (SIEMENS, USA).

Statistical Analysis

All collected date were carried out by least squares mean comparisons were evaluated using PDIFF option with the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). Individual gilt was used as the experimental unit and was analyzed 2 x 2 factorial arrangements. Differences and suggestive differences between treatments were considered at $P<0.05$ and $P<0.01$, respectively. The determination of tendency for all analysis was $P>0.05$ and $P<0.10$. The main plot was dietary energy levels and dietary lysine values of the gestating diets.

RESULTS AND DISCUSSION

Performance of Gestating Gilts

Table 2 shows the influence of different energy and lysine levels in gestation diet on bodyweight and body weight changes of sows. As shown in Table 2, no treatment effects were observed in body weight on gestation period despite increasing body weight of sows as time goes by. There were no effects of treatment on the body weight changes from breeding day to day 90 of gestation period ; however, body weight changes during gestation period (0~110d) were affected by dietary energy and lysine levels. The gilts fed high energy (3,140 kcal ME/kg) and high dietary lysine level (0.76%) in diet showed higher body weight gain (increasing 65.75 kg) than other gilts ($p<0.05$). Also, body weight gains during breeding day to farrowing day in T4 diet tended to be higher ($p<0.10$) when compared with the other treatments (48.38 kg vs. 41.90, 45.25, 42.93 kg, respectively). No effects of factors (energy level or dietary lysine level) were observed on body weight or its changes during whole gestation period of gilts. There were no interaction effects between energy and lysine in gestation diets. Kusina et al. (1999a, b) and Yang et al. (2009) reported that the maternal weight gains of sows were increased as the increasing dietary lysine levels. Cooper et al. (2001) demonstrated that the total lysine level (0.44%, 0.55%) did not affect total gestation body weight gain, regardless of parity. Mahan (1998) also reported that the dietary lysine concentration had no effect on sow body weights and weight gains during gestation period.

Pettigrew (1993) suggested pregnant sow should be fed 11 to 14 g of lysine/d to gain target amounts of lean tissue and prepared subsequent lactation. Dourmad et al. (2002) reported that N retention of gestation sow was maximized with 13.2 g of total lysine/d in the diet. Feed intake (FI) of gestating sows were 2.1

kg/d for all treatments. Then, the total lysine intakes of sow were 13.4, 15.5, 13.8 and 15.9 g/day, respectively. Because the dietary lysine level (0.64%) in the first treatment was already over the NRC (1998) dietary lysine requirements, this experiment showed there were no significant results for the maternal weight gains of gestation sows. The level of lysine in gestation diets showed tendency to affect gestation BW gain, when the diets were formulated such that one level was higher than the lysine level in NRC (1998). When the daily intake of total lysine was calculated (average of 13.6 and 15.7 g total lysine/d for low- and high-lysine diets, respectively), both diets were almost same as the current recommendations of NRC (2012) (11.0 to 17.5 g total basis lysine g/day). Diets were originally formulated based on NRC, 1998 recommendations. Thus, results of the present experiment confirm that the recommendations of NRC (2012), with respect to daily lysine intake, are adequate, even in animals producing at a high level.

Data for backfat thickness during gestation period are shown Table 3. Backfat thickness of primiparous sows for gestation period was not affected by energy lever or level of dietary lysine ($P>0.10$). Energy and lysine level of gestation diet did not affect backfact thickness changes from onset to the experiment for gilts until farrowing. Also there was not a energy level x lysine level in diet interaction for whole gestation period. However, treatment 4 sows on the high energy and lysine level of gestation diet gained numerically more backfat than the other treatments. Cooper et al. (2001) reported that there was a parity x gestation diet interaction ($P<0.05$); Parity 1 sows on the low-lysine gestation diet (0.50%) gained significantly less backfat than on the high-lysine gestation diet (0.64%) over the day 110 of gestation. Kusina et al. (1999a) demonstrated that backfat thickness of sows tended to decrease with increasing lysine intake during gestation. Mahan (1998) also reported that backfat thickness during gestation was higher ($P<0.01$) when sows were fed the 13% compared with the 16% protein diet, particularly after

parity 1. Also, Gill (2006) showed that excessive dietary lysine levels induced the decreasing backfat thickness. However, Yang et al. (2009) reported that an increasing dietary lysine levels during late gestation period resulted in higher backfat thickness. This result of Yang et al. (2009) indicated that higher lysine intake was more effective in increasing sow body fat content caused by catabolic stage in late gestation period than lower lysine intake. However, in this present experiment, higher lysine level (approximately 0.74%) in gestation diet did not show more backfat gain of gilts than low lysine level (approximately 0.64%). This demonstrated that the excess lysine did not affect to backfat gain of gilts for gestating period.

Reproductive Performance of Gilts

Table 4 showed the reproductive performance of pregnant gilts fed different energy and lysine level diets in gestation period. The number of piglet per litters and litter birth weight were numerically higher when gilts were fed low energy level (6,405 kcal ME/d) and 0.64 % lysine level diet during gestation period even though it did not show significant difference among treatments. Also, higher energy (6,594 kcal ME/d) or higher lysine (15.5g lysine/d) in gestation diet did not affect to reproductive performance of gilts for gestation period. Cooper et al. (2001) reported that the total number of piglets born and born alive per litter were not affected by lysine level ($p>0.10$) in gestation period. The study by Mahan (1998) also reported that the dietary lysine concentration provided during gestation period had no effect on litter size (total, stillbirths), or on litter birth weights ($p>0.15$).

Many articles already showed the relationship between energy level in gestation diet and reproductive performance (Dourmad, 1991; Coffey et al., 1994; Everts and Dekker, 1994; Mahan, 1998). Some articles about litter size at farrowing have reported adverse effects of increased gestation energy intake on the number of

piglets born alive (Buitrago et al., 1974; Libal and Wahstrom, 1977). However, in the present study, high gestation energy intakes were not related to a lower number of piglets born alive. Because gilts (parity 1) were still growing to mature size, high gestation energy intakes (6,570 ME kcal/d) did not affect reproductive performance. The recent high producing modern sows require much more lysine during gestation for maximizing both maternal and their fetal growth compared to conventional sows of previous decades. In the present study, there were no significant differences between two level of energy and two level of lysine in gestation diet. Consequently, this result demonstrated that the energy level (6,405 kcal ME/d) or the total lysine level (13.6 g lysine/d) is acceptable level to gilts for positive reproductive performance.

Blood Profiles of Gilts

Table 5 showed the blood profiles of gestation gilts fed different energy or lysine level diets in gestation period. The level of plasma glucose and insulin were not changed with high level of energy intake or lysine intake during gestation period. Glucose is very tightly controlled because it is a source of immediate energy for the tissues. Plasma glucose and insulin is positively correlated with feed or energy intake (Basset, 1974). However, in this experiment, the plasma glucose and insulin levels did not show the effect of energy intake even though feed intake was similar among treatments. Also, there were no interactions between energy and lysine in diets to gestation gilts on plasma glucose and insulin levels.

The concentration of blood urea nitrogen at day 35, 70 and 90 in gestation period numerically increased as the dietary lysine level increased. Kusina et al. (1999b) reported that increasing lysine intake during gestation increase ($p<0.05$) serum concentrations of blood urea nitrogen.

IMPLICATION

Consequently, 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 day 90 of gestation 7,775 to 8,182 kcal effective ME/kg during above day 90 of gestation. Also, total lysine requirements of NRC (2012) were 12.4 g/day during below day 90 of gestation 19.3 g/day during above day 90 of gestation. The requirements of lysine intake were increased than the requirements of NRC (1998). The energy level (6,396 to 6,588 kcal of ME/day) or dietary lysine level (13.4 to 15.9 g/day) of all treatments in this experiment were already almost same as NRC requirements (2012). Besides, a lower level of energy or lysine in gestation diet of gilt showed similar performance of growth and reproductive within normal range of those performances. Therefore, the energy level and dietary lysine level more than around 6,400 kcal of ME/day and 13.4 g/day, respectively, could be acceptable level on gilts with consideration of economy and environment.

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Table 1. The formula and chemical composition of gestation diet

Items, %	Total lysine level (%)			
	0.64(T1)	0.74(T2)	0.66(T3)	0.76(T4)
Corn	53.67	49.69	49.65	47.47
SBM, 44% CP	7.5	11.4	8.5	12.5
Beet pulp	3	3	4.1	3
Wheat bran	20	20	20	20
Coconut meal	3	3	3	3
Soybean hull	4.1	3.8	4.1	3
Tallow	3.1	3.5	5	5.4
Sugar molasess	3	3	3	3
L-Lysine HCl	0.1	0.1	0.11	0.11
DL-Methionine	-	0.01	-	0.03
L-Threonine	0.03	0.05	0.04	0.05
Limestone	0.79	0.79	0.79	0.79
DCP	0.96	0.91	0.96	0.9
Salt	0.35	0.35	0.35	0.35
MgO	0.11	0.11	0.11	0.11
Vit.Mix[a]	0.1	0.1	0.1	0.1
Min.Mix[b]	0.19	0.19	0.19	0.19
Chemical compositions(Calculated analysis)				
ME(kcal/kg)	3,045.45	3,059.24	3,123.85	3,137.27
Protein(%)	12.33	13.68	12.53	13.91
Fat(%)	6.34	6.63	8.14	8.43
Lysine(%)	0.64	0.74	0.66	0.76
Methionine(%)	0.21	0.23	0.21	0.25
Ca(%)	0.65	0.65	0.65	0.65
Total P(%)	0.64	0.65	0.64	0.64

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

[b] Supplied per kg diet: Cu, 55mg; Fe, 75mg; I, 250ug; Mn, 20mg; Se, 100ug; Zn, 30mg.

Table 2. Influence of different energy and lysine level in gestation diets on body weight, body weight changes of gilts.

Item	T1[1]	T2	T3	T4	SEM*	P-value		
						ME	Lys	MExLys
Body weight at pregnancy(kg)								
Breeding day	142.00	139.25	140.14	135.63	5.70	0.685	0.924	0.610
Day 15	152.80	146.18	147.93	146.00	5.75	0.597	0.687	0.474
Day 35	162.30	157.75	157.07	156.5	5.87	0.243	0.516	0.307
Day 70	181.90	175.5	178.21	180.13	7.58	1.000	0.798	0.132
Day 90	189.70	182.38	186.36	188.5	7.43	0.792	0.674	0.096
Day 110	199.40	193.88	195.36	201.38	7.95	0.873	0.792	0.086
Farrowing day	183.90	184.50	183.07	184.00	7.02	0.763	0.414	0.520
Body weight changes at pregnancy(kg)								
0~15 day	10.80	6.93	7.79	10.38	3.17	0.984	0.615	0.495
0~35 day	20.30	18.5	16.93	20.88	3.56	0.953	0.654	0.971
0~70 day	39.90	36.25	38.07	44.50	6.05	0.644	0.996	0.566
0~90 day	47.70	43.13	46.21	52.88	6.26	0.566	0.876	0.567
0~110 day	57.40[b]	54.63[b]	55.21[b]	65.75[a]	6.78	0.664	0.985	0.708
0~farrowing	41.90	45.25	42.93	48.38	4.29	0.694	0.399	0.858
110~farrowing	-15.50	-9.38	-12.29	-17.38	5.81	0.708	0.641	0.571

[1] T1=Low lys and energy, T2=High lys, low energy, T3=Low lys, high energy, T4=High lys and energy.

* Standard error of mean.

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

Table 3. Influence of different energy and lysine level in gestation diets on backfat and backfat changes of gilts.

Item	T1[1]	T2	T3	T4	SEM*	P-value		
						ME	Lys	MExLys
Backfat thickness at pregnancy(mm)								
Breeding day	17.60	20.13	18.57	16.63	3.75	0.600	0.741	0.285
Day 15	19.00	20.63	18.64	17.38	3.47	0.343	0.796	0.475
Day 35	19.80	20.38	19.29	18.13	3.41	0.473	0.922	0.805
Day 70	21.40	23.25	21.43	21.75	3.24	0.614	0.470	0.658
Day 90	23.00	24.50	23.93	24.25	2.59	0.832	0.403	0.732
Day 110	24.50	24.88	24.36	26.50	2.11	0.797	0.682	0.806
Farrowing day	22.60	23.38	23.07	25.88	2.39	0.579	0.545	0.748
Backfat changes at pregnancy(mm)								
0~15 day	1.40	0.50	0.07	0.75	0.91	0.096	0.676	0.064
0~35 day	2.20	0.25	0.71	1.50	1.65	0.704	0.559	0.048
0~70 day	3.80	3.13	2.86	5.13	2.35	0.902	0.617	0.279
0~90 day	5.40	4.38	5.36	7.63	3.16	0.424	0.748	0.321
0~110 day	6.90	4.75	5.79	9.88	3.63	0.643	0.974	0.585
0~farrowing	5.00	3.25	4.5	9.25	4.02	0.555	0.874	0.567
110~farrowing	-1.90	-1.50	-1.29	-0.63	1.14	0.245	0.245	0.677

[1] T1=Low lys and energy, T2=High lys, low energy, T3=Low lys, high energy, T4=High lys and energy.

* Standard error of mean.

Table 4. Influence of different energy and lysine level in gestation diets on reproductive performance.

Item	T1[1]	T2	T3	T4	SEM*	P-value		
						ME	Lys	MExLys
Farrowing performance								
No. of litters	6	5	7	5	-	-	-	-
No. born/litter	12.50	12.00	11.43	12.20	1.85	0.458	0.629	0.279
No. born alive/litter	12.33	11.80	11.43	11.80	1.72	0.458	0.758	0.319
No. stillbirths/litter	0.17	0.20	0.00	0.40	0.49	0.300	0.099	0.099
Piglet weight at farrowing(kg)								
Litter birth wt	17.21	14.24	15.62	15.22	2.72	0.792	0.262	0.160
Piglet birth wt	1.39	1.20	1.37	1.25	0.18	0.625	0.061	0.607

[1] T1=Low lys and energy, T2=High lys, low energy, T3=Low lys, high energy, T4=High lys and energy.

* Standard error of mean.

Table 5. Influence of different energy and lysine level in gestation diets on blood composition.

Item	T1[1]	T2	T3	T4	SEM*	P-value		
						ME	Lys	MExLys
Glucose(mg/dl)								
Day 15	74.8	57.5	56.3	65.0	17.19	0.547	0.423	0.343
Day 35	62.8	68.3	65.3	67.3	13.79	0.811	0.976	0.533
Day 70	74.5	71.3	60.0	83.8	12.21	0.992	0.850	0.997
Day 90	79.8^{a}	78.0^{a}	63.0^{b}	74.8^{a}	9.16	0.440	0.971	0.865
Day 110	82.0	87.8	83.8	77.3	10.47	0.692	0.850	0.262
Farrowing	106.2	96.0	99.7	104.5	12.54	0.842	0.654	0.313
Insulin(mlu/ml)								
Day 15	1.9	1.0	0.9	1.4	0.91	0.678	0.334	0.483
Day 35	2.0	1.0	2.5	1.6	1.23	0.881	0.877	0.422
Day 70	1.3	1.8	1.4	2.9	1.32	0.722	0.977	0.769
Day 90	3.4^{a}	2.2^{ab}	2.0^{ab}	1.2^{b}	1.31	0.386	0.610	0.596
Day 110	2.6	4.5	3.1	2.1	1.66	0.458	0.535	0.154
Farrowing	3.6	2.9	2.7	3.7	1.61	0.835	0.975	0.465
Protein(mg/ml)								
Day 15	6.1^{b}	8.0^{a}	7.8^{a}	7.7^{a}	0.94	0.024	0.018	0.002
Day 35	7.0	7.0	8.3	8.5	1.12	0.025	0.87	0.906
Day 70	8.4^{a}	7.1^{b}	6.5^{b}	9.0^{a}	1.35	0.949	0.837	0.537
Day 90	8.3	8.7	6.5	7.4	1.44	0.012	0.144	0.379
Day 110	7.6	8.4	7.6	7.8	0.80	0.510	0.394	0.413
Farrowing	7.5	7.7	6.6	7.2	0.83	0.024	0.153	0.475

[1] T1=Low lys and energy, T2=High lys, low energy, T3=Low lys, high energy, T4=High lys and energy.

* Standard error of mean.

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

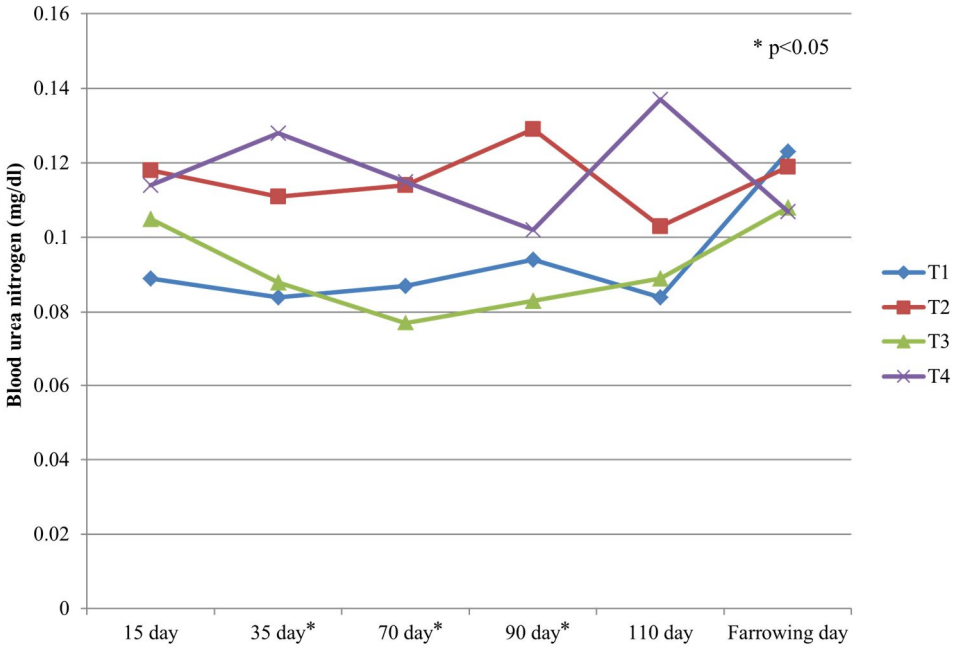

Figure 1. Influence of different energy and lysine level in gestation diets on blood urea nitrogen.

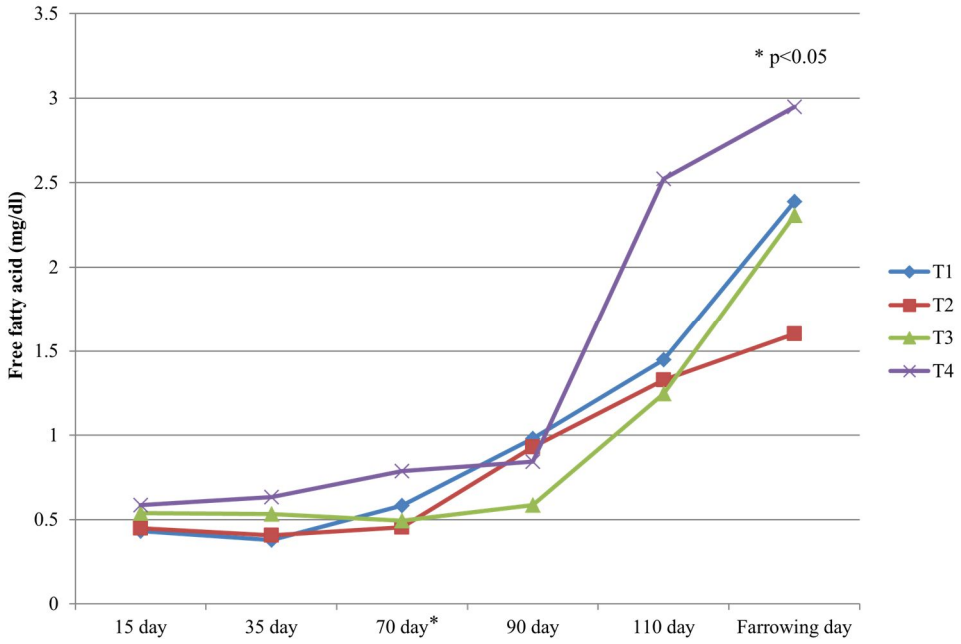

Figure 2. Influence of different energy and lysine level in gestation diets on free fatty acid in blood.

Chapter V : Effects of Different Levels of Fat Supplementation during Gestation on Reproductive Performance, Milk Composition and their Progeny Performance of Sows

ABSTRACT: The aim of this experiment was to determine the influence of different levels of fat during gestation on reproductive performance of sows. A total of 41 F1 multiparous sows (Yorkshire x Landrace) were allotted to 4 treatments by completely randomized design. During gestation, sows were fed different treatments containing either 1, 2, 3 or 4% of soybean oil but all sows were fed the same diet containing 1% soybean oil during lactation. There were no significant differences in body weight and backfat thickness of sows at day 110 of postcoitum and day 21 of postpartum regardless of dietary fat during gestation. Although the change in body weight of gestating sows were similar among treatment groups, the lowest backfat thickness change at day 110 of postcoitum was observed when sows were fed 3% soybean oil treatment diet ($P<0.05$). The litter size, total born live, birth weight and weight gain of nursing piglets were not influenced by the inclusion level of soybean oil during gestation. Colostrum had no response to increased fat level of gestation diet, especially fat concentration. The mortality of nursing pigs tended to be higher as dietary fat was increased. No effects were observed in weaning to estrus interval by the different supplementation levels of soybean oil. In economic analysis of gestation feed, the difference price of total gestation feed cost (per sow) is 7.92$ between 1% fat level and 4% fat level of gestation diet. These results demonstrated that higher levels of fat in sow′s diet did not show any beneficial effect on in reproductive performance of sows and fat concentration of colosturm was not improved by dietary fat supplementation in gestating sow’s diet.

Key words: Fat Level, Sow, Reproductive Performance, Litter Growth, Mortality, WEI

INTRODUCTION

For several decades, swine industry has advanced and changed through the higher sow productivity and improved lean genotype. And these changes are likely to continue. Thus, the modern hyper-prolific sow has larger litters of progenies, which puts greater demands on her ability to produce high quantity and quality milk. The neonatal mortality of the piglets during lactation period is higher especially day 0 to 3 after birth by susceptibility to cold and hypoglycemia. The two important energy storage forms in the neonatal piglet are glycogen and fat (Pettigrew, 1981). Glycogen stores in the neonatal piglet are very low and gluconeogenesis is not sufficient, thus glycogen in liver and skeletal muscle is depleted very quickly and may induce hypoglycemia (Mersmann,1974; Swiatek et al., 1968). Also, the newborn piglet's carcass contains only about 2% fat (Seerley et al., 1974), and much of this is structural and therefore unavailable for use as energy (Mersmann, 1974). In this situation, the piglet can obtain the energy only from colostrum. Le Dividich et al. (1991) investigated the effect of colostral fat content on fat deposition and plasma concentrations of metabolites. According to this paper, body fat deposition and plasma free fatty acids increased with the amount of ingested fat, that more liver glycogen was lost in pigs given the low-fat level and that plasma concentrations of glucose and insulin also were lower in pigs fed low-fat colostrum. This result means that colostral fat is important as a major factor in the supply of energy and in glucose homeostasis in the neonatal pig. Cranwell and Moughan (1989) reported that the apparent digestibility of fat by suckling piglets is high (96%). In addition, Friend (1974) suggested that an increase body lipid content in piglets at weaning in association with increased milk fat content, thus indicating that most of the lipids from the milk are deposited in the piglet's body. Several researchers have suggested that adding fat to sow's diet during pre- and post-partum increases the fat content of the colostrums and milk. The improvement in litter

weight by adding fat to sows diets has also been associated with an increased concentration of fat content and energy in the sow's milk (Shurson et al., 1986; Tilton et al., 1999). The adding of fat to diet during gestation may increase piglet survival (Pettigrew, 1981). However, in some studies, the effects on sow and litter performance are variable. The articles written by Pettigrew et al., (1989) and Babinszky et al., (1992) did not show consistent effects on reducing the body weight loss of sows and increasing litter weight of piglets during lactation period. Also, no significant improvements of litter performance (Nelssen et al., 1985) or sow performance (sows live weight, backfat thickness) could be obtained with supplementation of fat to sows diets. Seerly(1984) suggested differences in results due to variations in design of experiments, litter sizes, length of feeding supplemental fat, amount of fat fed, and initial survival rates of particular herds. Thus our objectives were to examine the influence of different levels of fat during gestation on performance of sows, pre-weaning performance of the piglets and on milk compositions.

MATERIALS AND METHODS

Experimental Animals, Design and Diet

A total of 41 crossbred sows (Yorkshire x Landrace; Darby Genetics Inc. Anseong, South Korea) with an average BW of 230.4 ± 20.8 kg and a parity 6.3 ± 1.7 were used to 4 dietary treatments. All sows were allotted to 4 treatments based on body weight, backfat thickness and parity by completely randomized design (CRD). The gestation diets were formulated to different level of fat supplement to 1%, 2%, 3% and 4% respectively. The diet composition for gestation and lactation sows was presented in Table 1. A gestation diet based on corn-soybean meal was formulated to contain 3,297 kcal of ME/kg, 12.90% CP and 0.73% total lysine, according to the requirement estimates (NRC, 1998). Calculated values of crude fat

of each treatment were 4.77%, 4.88%, 5.42% and 6.84%, respectively. All sows received the same diet and water *ad libitum* during a 3-week lactation period.

Animal Managements and Measurements

All pregnant sows were housed in individual gestation stalls (2.4 x 0.65 m^2) equipped with a nipple drinker. All sows were provided experimental diets individually in twice a day to meet their metabolic requirements after mating to farrowing. Feed intake of pregnancy sows was 2.4kg/d regardless of treatments. All sows received the same diet as an *ad libitum* during a 3-week lactation period. Sows were checked for estrus twice a day and were served 3 times of artificial insemination (AI) with 12 h interval from the second standing estrus. Semen used for AI was provided by Darby Genetics Inc., (Choongju, Korea). BW and BF thickness of gestation sows were recorded on day 0, 15, 35, 70, 90 and 110. Ultra-sound (Renco Corp., Minneapolis, Lean-meter, USA) was used for measuring BF thickness at the P2 position (mean value from both side of the last rib and 65 mm away from the backbone). Pregnant sows were moved to farrowing house after washing with an antiseptic solution on day 110. They were individually housed in a plastic floored 1.8 x 2.5 m farrowing crate, providing a nipple water supplier to each sow and the litter. The ambient temperature in the farrowing house was maintained at 23°C, and a heating lamp was installed for nursery piglets. Sows had free access to lactation feed and water throughout 21 days of lactation period. The litter was provided with only breast-feeding without any other creep feed and was injected intramuscularly with 120 ml of gleptoferron (463mg/ml equivalent to elemental iron 200mg/ml; Gleptosil, Intervet Korea Ltd., Seoul, Korea) to prevent an anemia. Teeth and end tail of piglets were removed and their ears were notched within 24 h after parturition. Male piglets were castrated after 3 days age. After postpartum, individual birth BW of piglets and the number of total and born alive

piglets were measured. Body weight of individual piglets as well as BW and BF thickness of sows were measured after farrowing, day 7, 14 and 21 of lactation period. At the same time, colostrum and mature milk were also collected. To stimulate the milk secretion for collecting milk sample, 0.5 ml (5 IU) of oxytocin (10 IU/ml, Oxyvet inj., Bayer Animal Health Co., Suwon, Korea) was injected via ear vein. Around 40 ml of each milk sample were collected from all possible teats by hands and was stored at -20°C in a sterilized plastic conical tube. All sows were moved to gestation stalls as soon as all piglets were weaned, and then same amount of gestation feed was provided to all sows until wean-to-estrus interval (WEI) was recorded.

Chemical Analysis

The frozen milk samples were allowed to thaw at 4°C for the analysis of milk composition. Milk samples were analyzed for fat, crude protein, lactose and solid-not-fat contents by using a milk composition auto-analyzer (MikoScan FT20, FOSS Electric Co., Denmark).

Statistical Analysis

All collected date were carried out by least squares mean comparisons were evaluated using PDIFF option with the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). Differences and suggestive differences between treatments were considered at $P<0.05$ and $P<0.01$, respectively. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of level of fat supplement in gestation diets.

Results and discussions

Performance of Gestating Sows

As shown in Table 2, no treatment effects were observed in body weight on gestation periods. The margin of body weight gain among treatments from breeding to d 110 of sows was increased as fat level rising although no significant differences were observed. There were no effects of treatment on the backfat thickness during gestation period. However, for gestation period, the multiparous sows fed 4% level of fat diet showed higher backfat thickness changes (increasing 31% of backfat thickness) than sows fed 2% and 3% level of fat diet (increasing 23% and 9% of backfat thickness, respectively) ($p=0.002$). Also, there was quadratic effects on backfat thickness changes from breeding to d 110 of sows ($p<0.004$). No effects were observed on weaning to estrus interval by dietary fat levels in gestating diet. In result of gestation length of multiparous sows, 2% treatment showed the longest gestation length than other treatments ($p=0.029$, quadratic, $p<0.009$).

Performance of Lactating Sows

Data for BW and BF thickness during lactation period are shown Table 2. Body weight of multiparous sows at day 1 after farrowing was showed no significant difference between treatments. On the other hand, a linear increases as fat level of diet raised, on body weight of sows after farrowing ($p=0.027$) were observed. There were slightly significant linear or no quadratic effects on body weight of sows at weaning (day 21). Also, no significant differences were observed among treatments with respect to sow BW at weaning and the changes of BW during the entire lactation period. Backfat thickness at postpartum (day 1) (linear, $p<0.152$) and weaning (day 21) (linear, $p<0.116$) were not greater as the rate of fat supplement in gestation diet increased. In addition, backfat thickness changes of multiparous sows during lactation period were not influenced by an increasing

dietary fat level. Overall ADFI of sows during whole lactation period was not affected by dietary fat level on gestation diet as shown in table 4.

Litter characteristics

Data for the performance of their progeny in each treatment are shown in Table 3. Fat levels of gestation diet did not affect the total number of piglet born as well as that of born alive. Also the number of dead piglets at first week of lactation period and whole lactation period did not differ among treatments. However, piglets mortality of treatments (3% and 4% fat level in diet) for 1 week was numerically lower than treatments 1% and 2% fat level in diet (5.50%, 5.83% vs. 7.12%, 7.64%). On the other hand, treatments 1% and 2% showed numerically lower total mortality of piglets than treatments 3% and 4% (9.06%, 9.17% vs. 13.76%, 12.25%). No significant differences by fat levels were observed on the weaned piglets. Also, there were neither linear nor quadratic effects of treatments on the number of weaned piglets and total mortality. Litter weight and piglet weight during whole lactation period did not influenced by treatments. There was quadratic effects ($p<0.014$) of treatments on the piglets weight at birth (1.30kg, 1.44kg, 1.49kg and 1.28kg, respectively). The multiparous sows fed 3% fat level showed numerically higher litter weight and piglets weigh at birth than the others (18.52 kg and 1.49kg, respectively), although there was no significant differences among treatments. The group fed 2% fat level of feed during gestation period were numerically higher the changes of litter weight and piglets weight for lactation period than the other treatments (35.57kg and 3.52 kg, respectively).

Characteristics of colostrum and milk

Data for the compositions of colostrums and mature milk from sows were showed in Table 5. Colostrum collected within 12 h after postpartum had no

response to increased fat level of gestation diet. Also, a linear increases as fat level raised, on the percentage of fat in colostrum were not observed (linear $p<0.663$). Milk fat content was affected by fat level in the gestation diet at 2 weeks of lactation($p=0.033$); the sows fed 2 % fat level resulted in a higher milk fat percentage than the other treatments (5.59%, 6.81% 5.99% and 6.08%, respectively). During the whole lactation period, the percentage of protein, lactose and solid-not-fat in colostrums and milk were not influenced by treatments.

Economic analysis of gestation feed

Table 6 showed the effects of different levels of fat supplementation during gestation on economical efficiency of feed during gestation. There were same amount of total feed intake during gestation period among the treatments. Feed costs (US$/kg) of each treatments were increased as fat level increased of diet (0.30$, 0.31$, 0.32$ and 0.33$, respectively). Also, there was a trend of linear increases in total feed cost as fat level increased of diet. There was 10% of relative feed cost between treatment 1% and treatment 4%.

DISCUSSION

It is well known that unsaturated fatty acids are absorbed more efficiently than saturated fatty acids in pigs (Freeman et al., 1968). The digestibility of a particular supplemental fat source in pigs is influenced by the fatty acid composition of the total diet. The digestibility of fat from diets which have a ratio higher than 1.5 of unsaturated to saturated fatty acids is high (85~92%) compared to that of diets with lower ratio (Stahly, 1984). Vegetable oils contain more unsaturated fatty acids than animal fats which largely consist of saturated fatty acids. In present study, a soy bean oil, which is one of vegetable oil, was mainly used by a fat source in experimental diet. Results of body weight and its changes during gestation period from this experiment did not show significant differences among treatments. However, there were a trend that changes of body weight were higher as increased fat levels of gestation diets although same amount of energy intake were offered all sows of each treatments (linear effect, $p<0.140$). Also, a linear increases as fat level of diet raised, on body weight of sows after farrowing ($p=0.027$) were observed. It could be explained that body weight changes of multiparous sows were more affected by high unsaturated fatty acids of diet.

Insufficient lactation feed consumption may increase the mobilization of body reserves for milk production and the incidence of reproductive problem after weaning (Foxcroft et al., 1995). Nutrient intake affects litter size, the occurrence of reproductive failure, and the rapidity of returns to service after weaning through their effects on follicular development as well as rates of fertilization, implantation, and pregnancy (Koketsu et al., 1996). Energy intake during lactation is related to sow weight loss during lactation, (Elsely et al., 1968; O'Grady et al., 1975), thus low energy intake during lactation may contribute to delayed estrus following weaning. The results of this study showed that the increase in fat level of gestation diet could not affect the losses of body weight or backfat thickness as well as the

lactation ADFI. Besides, the number of days from weaning to estrus was not influenced by supplemental fat of gestation diet, although the interval as reduced from 5.11 d in 1% fat supplemented diets to 4.60 d in sows given 4% fat supplemented diets during gestation period. During lactation period, average daily feed intake and body weight loss of sows were not significant difference among all treatments. It means fat level of gestation diets did not affect feed intake of lactation period, consequentially, no difference in weaning to estrus interval showed among all treatments.

The article written by Pettigrew (1981) has shown that addition of dietary fat to the diets of sows during late gestation and (or) lactation increases milk production and fat content of colostrum and milk. This increase in colostral and milk fat concentration improves the survival rate among the piglets. In addition, several studies indicated an improved survival of piglets by feeding fat to sows before farrowing (Moser and Lewis, 1980; Drochner, 1989), as well as enhancement in piglet weight gain (Seerley et al., 1974; Boyd et al., 1978; Barbinszky et al., 1992; Tilton et al., 1999), and a decrease of the body loss of the sows during lactation period (Kornblum et al., 1991). However, the results of present study were in conflict with several studies. In present study, colostrum collected within 12 h after postpartum had no response to increased fat levels of gestation diet. In addition, a linear increases as fat level raised, on the percentage of fat in colostrum were not observed (linear effect, $p<0.663$). On the other hand, during 1 week after farrowing, the piglet mortality was numerically low on higher fat level treatments. Moreover, no significant differences were observed on litter weight and piglet weight for 1 week after farrowing. Those results mean that high fat level (4%) of gestation diet did not influence only fat concentration of colostrum but also a growth performance of their progeny. According to previous study (Pettigrew, 1981), the increases were significant when the dietary fat concentration

was at least 8% and was fed through both gestation and lactation. In this experiment, therefore, the 4% level of fat supplementation, which was the highest fat level among treatments, could be low to response to show the effects of addition fat to diet.

The cost of each treatment diets were 0.30 US $, 0.31 US $, 0.32 US $ and 0.33 US $ for the 1%, 2%, 3% and 4% fat level diets respectively. The feed cost elevation in 3% and 4% fat level diets was due to increase a soybean oil inclusion levels as much a decreasing corn levels into the diets. The elevation of fat level into the diets had no better results on the main performance parameters studied ie reproductive performance, lactation performance and WEI. However, the difference price of total feed cost (per sow) is 7.92$ between 1% fat level and 4% fat level of gestation diet. Consequently, 1% fat level in sow's diet showed the growth and lactation performance within normal range and positive effect on feed cost and economy.

IMPLICATIONS

From the present study, it can be indicated that increased fat level of gestation diet did not influence chemical compositions of colostum and milk, especially fat concentration. Higher fat levels of gestation diet had little impact on litter size and litter weight at birth in addition to the subsequent litter performance during the whole lactation period. Also, the mortality of nursing pigs and weaning to estrus interval were not decreased by higher dietary fat supplementation in gestating sow's diet. In conclusion, this result suggested that higher levels of fat in sow′s diet did not show any beneficial effect on reproductive performance of multiparous sows in high parity.

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Table 1. The formulas and chemical composition of experimental diets

Items, %	Gestation[1] : Treatments				Lactation[2]
	1%	2%	3%	4%	
Corn	74.84	69.77	63.08	57.41	67.42
SBM, 46% CP	15.36	14.64	14.22	13.60	24.62
Wheat bran	0.00	4.88	9.50	14.33	2.50
Cassava meal	4.65	4.64	6.20	6.76	-
Sugar molasses	-	-	-	-	1.05
Soy oil	1.00	2.00	3.00	4.00	1.00
L-Lysine HCl	0.19	0.19	0.18	0.17	0.38
DCP	2.54	2.38	2.24	2.08	1.50
Limestone	0.82	0.90	0.98	1.05	0.78
Vita. mix[3]	0.20	0.20	0.20	0.20	0.20
Min. mix[4]	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Choline chloride, 50%	-	-	-	-	0.15
Total	100.00	100.00	100.00	100.00	100.00
Chemical compositions (calculated values, %)					
ME, kcal/kg	3297.92	3297.92	3297.92	3297.92	3265.00
Crude protein, %	12.90	12.90	12.90	12.90	16.80
Lysine, %	0.73	0.73	0.73	0.73	1.08
Methionine, %	0.23	0.23	0.22	0.22	0.28
Calcium, %	0.90	0.90	0.90	0.90	0.90
Total phosphorus, %	0.70	0.70	0.70	0.70	0.70
Chemical compositions (analyzed values, %)					
Crude Fat, %	4.77	4.88	5.42	6.84	

[1] Gestation diets was daily provided in 2 separated meals by the same amount at 0800 and 1600h. All sows received 2.4 kg/d during the whole gestation period.

[2] Sows had free access to lactation diet throughout 3 weeks of lactation period.

[3] Provided the following quantities of vitamins per kg of complete diet : vitamin A, 10,000 IU; vitamin D_3, 1,500 IU; vitamin E, 35 IU; vitamin K_3, 3mg; pantothenic acid, 10mg; niacin, 20 mg; biotin, 50 ug; vitamin B_{12}, 15ug; folic acid, 500 ug; vitamin B_2, 4mg; vitamin B_6, 3mg.

[4] Provided the following quantities of minerals per kg of complete diet : Cu, 55 mg; Fe, 75 mg; I, 250 mg; Mn, 20 mg;

Se, 100 ug; Zn, 30 mg ; Co, 250 mg.

Table 2. Effects of different levels of fat supplementation during gestation on body weight, backfat thickness and weaning to estrus interval of multiparous sows

Items	Treatments[1]				SEM[2]	P-value	
	1%	2%	3%	4%		Linear	Quad
No. of sows	9	11	9	12	-	-	-
Avg. parity	6.22	6	6.56	6.5	-	-	-
Live body weight, kg							
Breeding	228.28	227.14	232.67	233.42	3.26	0.411	0.792
Day 110 postcoitum	273.11	273.5	281.5	284.33	3.09	0.141	0.737
Breeding to 110d	44.83	46.36	48.83	50.92	1.68	0.140	0.882
Day 1 postpartum*	256.06	257.23	264.89	270.67	3.06	0.027	0.534
Day 21 postpartum	245.11	250.73	257.17	259.33	3.02	0.054	0.86
Farrowing to 21d	-10.94	-6.5	-7.72	-11.33	1.35	0.585	0.113
Backfat thickness, mm							
Breeding	14.5	16.18	19.22	17.63	0.80	0.100	0.420
Day 110 postcoitum	18.44	20	21	23.21	0.96	0.069	0.781
Breeding to 110d**	3.94^{bc}	3.82^{b}	1.78^{a}	5.58^{c}	0.42	0.261	0.004
Day 1 postpartum	19.72	20.45	21.33	22.96	0.99	0.152	0.595
Day 21 postpartum	18.5	19.5	20.78	21.75	0.91	0.116	0.775
Farrowing to 21d	-1.22	-0.95	-0.56	-1.21	0.26	0.957	0.303
Weaning to estrus interval, d	5.11	4.81	4.48	4.6	0.16	0.330	0.529

[1] Level of fat supplementation in gestation diet.

[2] Standard error of mean.

[a,b,c] Means with different superscripts significant difference (p=0.002).

[A,B] Means with different superscripts significant difference (p=0.029).

* Linear effect.

** Quadratic effect.

Table 3. Effects of different levels of fat supplementation during gestation on litter size and litter weight during lactation period

Items	Treatments[1]				SEM[2]	P-value	
	1%	2%	3%	4%		Linear	Quad
No. of sows	9	11	9	12			
Litter size							
Total born	13.56	12.36	12.56	13.92	1.19	0.222	0.057
Born alive	11.22	11.55	10.78	11.92	1.02	0.224	0.355
After cross-fostering	11.67	11.64	11.89	12.08	0.33	0.101	0.629
Dead for 1 week, head	0.89	0.91	0.67	0.75	0.42	0.837	0.835
Total dead, head	1.11	1.09	1.67	1.50	0.48	0.446	0.751
Weaned pig (21day)	10.56	10.55	10.22	10.58	0.41	0.826	0.710
Mortality for 1 week	7.12	7.64	5.50	5.83	3.39	0.768	0.944
Total mortality	9.06	9.17	13.76	12.25	3.93	0.486	0.680
Litter weight, kg							
At birth	17.07	17.74	18.52	17.61	0.57	0.761	0.521
After cross-fostering**	16.32	17.41	18.02	16.40	0.36	0.886	0.036
Day 7	24.75	26.88	27.46	25.35	0.62	0.731	0.078
Day 14	38.24	40.25	39.80	38.39	1.01	0.953	0.441
Day 21	50.80	52.99	53.29	51.45	1.37	0.941	0.477
Piglets weight, kg							
At birth**	1.30	1.44	1.49	1.28	0.31	0.943	0.014
After cross-fostering**	1.40	1.49	1.51	1.36	0.26	0.792	0.023
Day 7	2.29	2.49	2.44	2.25	0.46	0.807	0.064
Day 14	3.57	3.80	3.75	3.59	0.69	0.872	0.237
Day 21	4.81	5.02	5.21	4.91	1.01	0.499	0.244
Weight change during lactation, kg							
Litter weight	34.48	35.57	35.27	35.05	1.24	0.966	0.827
Piglets weight	3.40	3.52	3.70	3.54	0.95	0.446	0.518

[1] Level of fat supplementation in gestation diet.

[2] Standard error of mean.

** Quadratic effect.

Table 4. Effects of different levels of fat supplementation during gestation on average daily feed intake of multiparous sows during lactation period

Item	Treatments[1]				SEM[2]	P-value	
	1%	2%	3%	4%		Linear	Quad
Average daily feed intake, g							
1st week	4336	4392	4953	4423	134.31	0.492	0.295
2nd week	4676	4590	4331	4453	135.42	0.434	0.797
3rd week	5224	5172	5250	4987	166.72	0.570	0.678
Overall (0-21 days)	4745	4718	4845	4621	93.83	0.677	0.567

[1] Level of fat supplementation in gestation diet.

[2] Standard error of mean.

Table 5. Effect of different levels of fat supplementation during gestation on milk composition of multiparous sows during lactation period

Items	Treatments[1]					P-value	
	1%	2%	3%	4%	SEM[2]	Linear	Quad
Fat, (%)							
Colostrum	6.22	4.87	6.57	6.15	0.48	0.663	0.543
Day 7 [3]	5.66	7.11	6.27	6.31	0.23	0.579	0.133
Day 14 **	5.59^{B}	6.81^{A}	5.99^{B}	6.08^{AB}	0.15	0.556	0.041
Day 21	5.50	6.01	6.39	5.87	0.16	0.288	0.112
Protein, (%)							
Colostrum	10.57	11.52	8.99	9.18	0.59	0.138	0.687
Day 7	4.98	5.08	4.70	4.87	0.07	0.343	0.815
Day 14	4.65	4.97	4.73	4.68	0.05	0.725	0.109
Day 21	4.89	4.85	4.75	5.07	0.06	0.526	0.238
Lactose, (%)							
Colostrum	3.36	3.21	3.68	3.59	0.12	0.264	0.882
Day 7	5.43	5.36	5.54	5.45	0.03	0.555	0.933
Day 14	5.78	5.62	5.73	5.76	0.03	0.829	0.172
Day 21	5.72	5.75	5.77	5.65	0.02	0.479	0.252
Solid-not-fat, (%)							
Colostrum	13.52	14.29	12.41	12.55	0.42	0.125	0.625
Day 7	10.41	10.35	10.19	10.28	0.06	0.337	0.538
Day 14	10.45	10.55	10.50	10.44	0.03	0.809	0.347
Day 21	10.59	10.55	10.48	10.72	0.04	0.545	0.236

[1] Level of fat supplementation in gestation diet.

[2] Standard error of mean.

[3] After farrowing days.

[A,B] Means with different superscripts significant difference (p=0.033).

** Quadratic effect.

Table 6. Effects of different levels of fat supplementation during gestation on economical efficiency of feed during gestation

Item	Treatments[1]			
	1%	2%	3%	4%
Feed cost[2] during gestation				
Total amount of feed intake, kg	264.0	264.0	264.0	264.0
Feed cost, US$/kg	0.30	0.31	0.32	0.33
Total feed cost, US$/sow	79.20	81.84	84.48	87.12
Relative feed cost, %	100.0	103.3	106.6	110.0

[1] Level of fat supplementation in gestation diet.

[2] Feed cost of gestation diet (breeding to 110 d).

Chapter VI. Overall Conclusion

Evaluating alternative nutritional manipulation of pig diet is the most biologically, financially and environmentally effective way of pig industry. Although many research for pig diet have been conducted over more than several decades, limited information is still available and the results are inconsistent to evaluate alternative nutritional manipulation. Therefore, three experiments were conducted to investigate 1) effects of benzoic acid supplementation on the performance, nutrient digestibility, blood profile and ammonia gas emission in weaning and growing pigs, 2) influence of different energy and dietary lysine levels in gestation diets on reproductive performance, blood composition and growth of gilts, and 3) effects of different levels of fat supplementation during gestation on reproductive performance, milk composition and their progeny performance of sows.

During weaning phase I (0-2 weeks), Pcon and Pove treatment groups showed significant differences in BW, ADG, and G:F ratio compared to Ncon ($P<0.05$). Pig fed with an antibiotic diet (Pcon, PoVe) showed the highest growth performance during first 2 weeks, followed by pig fed a diet supplemented with benzoic acid only, while Ncon pig had the lowest growth rate throughout the weaning phase. The effects of treatments during the weaning period affected the growth performance of the subsequent growing period (5th-11th weeks). In treatment groups Pcon, NeVe and PoVe, ADG was increased by 6.78%, 5.65% and 8.62%, respectively, compared with treatment Ncon. In addition, the tendency that pig that received antibiotic with benzoic acid supplementation has the highest numerical value of ADG as observed in the weaning period, was also present in the growth period. Over the entire experimental period, antibiotic, benzoic acid, and antibiotic with benzoic acid supplementations showed tendencies toward increasing

body weight, average daily gain, and average feed intake in treated pig.

In the weaning period trial, supplemented benzoic acid groups showed a trend to a lower urine pH than the values in treatments Ncon and Pcon; however, the trend was not significant (P=0.069). Also, a nearly significantly lower urine pH was observed in treatment PoVe in the growing period trial (P=0.059). In this experiment, the pH of feces tended to be lower in the NeVe and PoVe treatments supplemented with benzoic acid than in the treatments without benzoic acid by 9.01% and 4.05%, respectively. Based on these results, when benzoic acid is supplemented in pig diet, ammonia gas emission could be reduced through a tendency toward a lowering of urine pH.

According to results of experiment 2, body weight changes during whole gestation period (day 0~110) was affected by dietary energy and lysine levels. The gilts fed high energy (3,140 kcal ME/kg) and high dietary lysine level (0.76%) in diet showed higher body weight gain (increasing 65.75 kg) than other gilts ($p<0.05$). Also, body weight gains during breeding day to farrowing day in high energy and lysine supplementation diet tended to be higher ($p<0.10$) when compared with the other treatments (48.38 kg vs. 41.90, 45.25, 42.93 kg, respectively). No effects of factors (energy level or dietary lysine level) were observed on body weight or its changes during whole gestation period of gilts. Gestation energy level or lysine level did not affect backfact thickness changes from onset to the experiment for gilts until farrowing. Also there was not a energy level x lysine level in diet interaction for whole gestation period. The number of piglet per litters and litter birth weight were numerically higher when gilts were fed low energy level (6,405 kcal ME/d) and 0.64 % lysine level diet during gestation period even though it didn't show significant difference among treatments. Also, higher energy (6,594 kcal ME/d) or higher lysine (15.5g lysine/d) in gestation diet did not affect to reproductive performance of gilts for gestation period.

On the other side, the margin of body weight gain among treatments from breeding to day 110 of sows was increased as fat level rising although no significant differences were observed. There were no effects of treatment on the backfat thickness during gestation period. However, for gestation period, the multiparous sows fed 4% level of fat diet showed higher backfat thickness changes (increasing 31% of backfat thickness) than sows fed 2% and 3% level of fat diet (increasing 23% and 9% of backfat thickness, respectively) ($p=0.002$). Also, no significant differences were observed among treatments with respect to sow BW at weaning and the changes of BW during the entire lactation period. In addition, backfat thickness changes of multiparous sows during lactation period were not influenced by an increasing dietary fat level. Overall ADFI of sows during whole lactation period was not affected by dietary fat level on gestation diet. Fat levels of gestation diet did not affect the total number of piglet born as well as that of born alive. Also the number of dead piglets at first week of lactation period and whole lactation period did not differ among treatments. No significant differences by fat levels were observed on the weaned piglets. Also, there were neither linear nor quadratic effects of treatments on the number of weaned piglets and total mortality. Litter weight and piglet weight during whole lactation period did not influenced by treatments. Colostrum collected within 12 h after postpartum had no response to increased fat level of gestation diet. Also, a linear increases as fat level raised, on the percentage of fat in colostrum were not observed (linear $p<0.663$). During the whole lactation period, the percentage of protein, lactose and solid-not-fat in colostrums and milk were not influenced by treatments. There were same amount of total feed intake during gestation period among the treatments. There was a trend of linear increases in total feed cost as fat level of diet raise. There was 10% of relative feed cost between treatment 1% and treatment 4%.

Consequently, these nutritional manipulations with benzoic acid, dietary

energy level, dietary lysine level and dietary fat level in swine diets could enhance swine productivity including physiological responses, economical benefit and pollution prevention in swine production.

Chapter VII. Summary in Korean

본 실험은 돼지 사료의 영양적 조절이 이유 자돈 및 모돈의 생리와 생산성에 미치는 영향을 평가하기 위해 수행되었다. 이유 및 육성돈 초기 사료 내 벤조익산의 첨가가 성장성적, 영양소 소화율, 혈액 성상 및 암모니아 가스 발생에 미치는 영향, 임신돈 사료 내 에너지와 라이신 함량이 번식성적 및 혈액 성상에 미치는 영향 그리고 임신돈 사료 내 지방 첨가 수준에 따라 번식 성적, 모유 성분 및 포유 자돈에 미치는 영향등에 관한 실험이 수행되었다.

Experiment I. The Effect of VevoVitall® Supplementation on the Performance, Nutrient Digestibility, Blood Profile and Ammonia Gas Emission in Weaning and Growing Pigs

본 실험은 이유 자돈 및 육성돈 초기 사료 내 벤조익산의 첨가가 사양 성적, 영양소 소화율, 혈액 성상 및 암모니아 가스 발생에 미치는 영향을 검증하기 위해 수행되었다. 총 128두의 삼원교잡종 ([Landrace X Yorkshire] x Duroc)자돈을 성별과 체중을 고려하여 이유(이유일령24±3일)시에 전체 4처리 8반복, 반복당 4두씩 난괴법(RCBD; Randomized Complete Block Design)으로 배치하였다. 실험 처리구는 1)Ncon (basal diet), 2)Pcon (basal diet + 항생제 0.12%), 3)NeVe (Ncon + benzoic acid 0.5%), 4)PoVe (Pcon + benzoic acid 0.5%)로 구성되었고, 사료 내 실험처리는 이유자돈 5주와 육성기 6주로 총 11주 동안 처리되었다. 이유자돈

기간 내 항생제와 벤조익산을 처리한 3개의 처리구는 Ncon 처리구보다 체중과 일당 증체량 그리고 일당 섭취에서 보다 높은 결과를 보여주었다. 하지만, 벤조익산과 항생제를 처리한 3개의 처리구 간의 통계적인 유의적 차이는 나타나지 않았다. 이유 자돈 기간 내 나타났던 항생제와 벤조익산의 사료내 첨가가 돼지 성장에 미치는 영향은 육성기에도 유의적인 차이는 보이지 않았지만 긍정적인 경향을 보였다. 또한 항생제와 벤조익산간의 상호작용 또는 상승작용 효과를 조사하였다. 항생제와 벤조익산을 같이 처리한 PoVe 처리구에서 다른 처리보다 높은 성장율을 보여주었음에도 불구하고, 통계적으로 상호작용 또는 상승작용 효과가 나타나지 않았다. 자돈과 육성돈의 소화율 측정 시험 결과에서는 처리간의 어떠한 차이도 나타나지 않았다. 또한 혈중 요소태 함량, 질소 축적율에서도 처리간 차이는 보이지 않았다. 벤조익산의 효과를 연구하기 위해 자돈구간과 육성구간에서 각각 요 내의 pH을 측정하였다. 자돈 구간 내 요의 pH는 벤조익산을 처리한 PoVe와 NeVe 처리구에서 다른 처리구 보다 낮게 측정되었다($p=0.069$). 또한 육성기 구간에서도 벤조익산과 항생제를 처리한 PoVe 처리구에서 다른 처리구에 비해 낮은 pH 결과를 보여주었다($p=0.059$). 분 내 pH의 경우 통계적인 유의적인 차이는 아니었지만, 벤조익산을 처리한 NeVe, PoVe 처리구에서 다른 처리구에 비해 각각 9.01%, 4.05% 낮은 결과를 보여주었다. 따라서, 결론적으로 자돈과 육성구간 돼지 사료 내 벤조익산의 첨가는 성장율 개선과 요 내 pH를 낮추는데 긍정적인 효과를 보여주었다.

Experiment II. Influence of Different Energy and Lysine Levels in

Gestation Diets on Reproductive Performance, Blood Composition and Growth of Gilts

본 실험은 임신모돈 사료 내 에너지 함량과 라이신 함량이 모돈의 번식 성적 및 생리적 반응에 미치는 영향을 조사하기 위하여 수행되었다. 30두의 F_1 임신모돈 (Yorkshire × Landrace)을 공시하여 종부 후에 체중 및 등지방 두께를 고려하여 2 X 2 요인 설계 방법에 따라 배치하였다. 요인은 에너지 수준 (3,050 및 3,140 kcal of ME/kg)과 라이신 수준(사료내 0.64%와 0.74%)이었으며, 각각의 임신돈에게 실험사료는 각각 2.1kg/일씩 공급되었다. 일일 라이신 총 섭취량은 각각 처리별로 13.4, 15.5, 13.8 그리고 15.9g 이었다. 임신 기간 내 각 시기별 체중은 처리가 유의적인 차이는 보이지 않았지만, 전체 임신 기간 내(0~110일) 임신돈의 체중 증체량은 실험 사료 내 높은 에너지 수준과 높은 라이신 수준을 첨가한 처리구에서 다른 처리구에 비해 높은 결과를 보여주었다($p<0.05$). 하지만 임신기간 내 모돈의 체중과 증체량은 사료 내 에너지 함량 또는 라이신 첨가 수준에 대한 영향을 보여주지 않았다. 또한 임신돈 등지방 두께 변화도 각 처리에 대한 영향을 보여주지 않았다. 분만 성적의 경우, 낮은 에너지와 라이신 함량 처리구에서 복당 산자수와 생시체중이 다른 처리구에 비해 수치적으로 다소 높았다. 또한 임신돈 사료 내 높은 수준의 에너지와 라이신 수준은 번식 성적에 영향을 주지 않았다. 혈장 내 글루코스와 인슐린 수준은 임신돈 사료 내 높은 수준의 에너지 함량과 라이신 수준에도 다른 처리구와 차이는 나타나지 않았다. 따라서, 임신돈 사료 내 6,400 kcal of ME/일 의 에너지 수준과 13.4 g/일의 라이신 수준

은 모돈의 성장과 번식 성적에 어떠한 부정적인 효과 없이 모돈에게 필요한 요구량을 충족시킨다고 사료된다.

Experiment III. Effects of Different Levels of Fat Supplementation during Gestation on Reproductive Performance, Milk Composition and Their progeny Performance of Sows

본 실험은 임신돈 사료 내 지방의 첨가수준이 모돈의 번식 성적, 유성분 그리고 포유자돈의 성장에 미치는 영향을 연구하기 위해 수행되었다. 총 41두의 F_1 임신모돈 (Yorkshire × Landrace)을 공시하여 종부 후에 체중 및 등지방 두께 그리고 산차를 고려하여 완전임의배치법으로 4개의 처리구별로 배치하였다. 실험 처리는 각 처리별로 대두유를 1,2,3 그리고 4%를 임신돈 사료 내 첨가하였고, 포유돈 사료는 사료 내 1%의 대두유 첨가를 동일하게 처리하였다. 임신기 기간내 각 처리별 모돈들의 체중에는 처리에 대한 영향이 나타나지 않았다. 임신기간 내 각각의 처리간의 체중 변화량은 비슷한 경향을 보였지만, 임신 110일령에 대두유 3%를 첨가한 처리구 그룹에서 다른 처리구 그룹들 보다 적은 등지방 두께 변화량을 보였다($p<0.05$). 임신돈 사료 내 대두유 첨가 수준에 따른 복당 산자수, 생시 두수, 생시 체중 그리고 포유 자돈 증체량은 각 처리간의 통계적인 유의차를 보여주지 않았다. 초유의 성분의 경우 특히 지방 함량은 각 시험 처리간의 차이가 나타나지 않았다. 또한 재귀발정일의 경우에도 각 처리간의 유의적인 차이가 나타나지 않았다. 임신돈 사료의 경제석 분석 결과는 대두유 1% 첨가 처리구와 4% 첨가 처리구의

모돈 두당 임신기 전체 사료비 차이는 약 $7.92로 대두유 1%첨가 처리구가 경제적인 측면에서 좋은 결과를 나타내었다. 위의 모든 결과를 토대로 임신돈 사료 내 4% 수준의 지방(대두유) 첨가는 번식 성적 그리고 모유 내 지방함량에 어떠한 긍정적인 영향을 보여주지 않았다.