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# Effect of Dietary Crude Protein Reduction in Weaning Pigs

## 사료 내 조단백질 함량의 감소가 자돈에 미치는 영향에 관한 연구

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이 논문을 농학석사 학위논문으로 제출함

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# Summary

This experiment was conducted to evaluate the effect of dietary crude protein reduction on growth performance, blood profiles, and nutrient digestibility in weaning pigs. A total of 128 crossbred piglets ([Yorkshire × Landrace] × Duroc), weaned at 21 ± 3 d of age with average initial body weight of 7.68 ± 0.2 kg were used in this experiment during 5 weeks. Weaning pigs were assigned to one of 4 treatments in a randomized complete block (RCB) design in 8 replicates with 4 pigs per pen. Control diet had same level of dietary crude protein as NRC (1998) recommended, and 2% of dietary crude protein was reduced from 2% to 6% less then NRC recommendation. Three phase feeding programs were used in this experiment (phase I, d 0 to 7; phase II, d 7 to 21; phase III, d 21 to 35). In growth performance, reduction of dietary crude protein resulted in decrease of growth performance, but 2% reduction of dietary crude protein treatment showed significantly same rows with control diet ($P$<0.05). Pigs fed control diet showed the highest serum blood urea nitrogen (BUN) concentration, but insulin-like growth factor-1 (IGF-1) level was decreased by dietary crude protein reduction (linear, $P$<0.05). Sixteen crossbred barrows (4 pigs per treatment) averaging 16.28 ± 1.82 kg body weight were alloted in apparent nutrient digestibility. Dry matter digestibility was increased by dietary crude protein reduction (linear, $P$<0.05; quadratic $P$<0.01), and 4% reduction of dietary crude protein treatment showed significantly higher dry matter digestibility than other treatment ($P$<0.01). Four percent reduction of dietary crude protein treatment showed significantly power total nitrogen excretion than other treatment ($P$<0.01). Also, dietary crude protein digestibility was lowered by dietary crude protein digestibility (linear, $P$<0.05), and crude ash

digestibility was increased by dietary crude protein reduction (linear, $P<0.05$; quadratic, $P<0.05$). Crude fat digestibility showed some tendency of increased than decreased, and 4% reduction of dietary crude protein treatment showed the highest crude fat digestibility (quadratic, $P=0.08$). In nitrogen retention, fecal nitrogen was decreased by dietary crude protein reduction (linear, $P<0.05$), and also, urinal nitrogen showed decreased tendency by dietary crude protein reduction ($P=0.067$). Consequently, reduction of dietary crude protein level in weaning pig diet reduce growth performance, but improve nutrient digestibility. And 4% dietary crude protein reduction could be acceptable for commercial weaning pigs feed with considering impact of environmental pollution.

**Key words** : Crude protein, Nitrogen excretion, Weaning pigs, Growth performance, Diarrhea

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## Ⅱ. Experiment

# List of Abbreviation

| | | |
|---|---|---|
| ADFI | : | Average daily feed intake |
| ADG | : | Average daily gain |
| AOAC | : | Association of official analytical chemists |
| BW | : | Body weight |
| BUN | : | Blood urea nitrogen |
| Ca | : | Calcium |
| CF | : | Crude fat |
| CP | : | Crude protein |
| CRD | : | Completely randomized design |
| DM | : | Dry matter |
| FE | : | Feed efficiency |
| G/F | : | Gain to feed ratio |
| GLM | : | General linear model |
| LSD | : | Least significantly difference |
| LPS | : | Lipopolysaccharide |
| MCP | : | Mono-calcium phosphate |
| ME | : | Metabolizable energy |
| N | : | Nitrogen |
| NRC | : | National Research Council |
| P | : | Phosphorus |
| RCB | : | Randomized complete block |
| SAS | : | Statistical Analysis System |
| SBM | : | Soybean meal |
| SPC | : | Soy protein concentrate |

# I. Introduction

Weaning gave the piglets a lot of stress which is related with environment, social, physiology and abrupt change of feed. These stressful circumstance induced growth lag and nutritional disease in young pigs. Abrupt change of feed, among these stress, from sow's milk to solid ingredients in the diet such as corn and soybean meal could be the most critical in digestive immaturity of weaning pigs. Etheridge et al. (1984) reported that the fermentation in the lower bowel was significantly greater in pigs fed cereal diets when compared to pigs fed only sow's milk, and feal osmolarity and volatile fatty acid concentrations were both increased. This results demonstrated that undigested feed could increase the fermentation in large intestine and cause occurrence of diarrhea.

The content of dietary CP, individual AA, or both might affect the formation and concentrations of metabolites resulting from microbial fermentation (Hobbs et al., 1996). High dietary CP concentration, as is common in diets for early-weaned pigs, but it may increase microbial fermentation of undigested protein, and encourage proliferation of pathogenic bacteria in the gastrointestinal tract (Ball and Aherne, 1987). Bacterial fermentation of undigested protein produces VFA and potentially toxic substances such as ammonia and amines that can reduce growth of weaned piglets (Gaskins, 2000). The incidence of diarrhea at weaning in pigs has been associated with increased production of amines and ammonia (Dong et al. 1996). Diets for weaning pigs usually contain high levels of protein, which may encourage proliferation of pathogenic bacteria in the gastrointestinal tract (Ball and Aherne, 1987). High dietary protein content of weaning pigs diets may increase the incidence of postweaning diarrhea, results in poor performance. It

is careful to speculate that reducing dietary protein supply with appropriate AA supplementation can reduce the amount of substrate for bacterial proliferation in the gastro-intestinal tract (GIT) and this would be economical benefit to farm family.

A new Korean agricultural law was enacted in 2012. This law consists limiting total N concentration in livestock effluent as 250ppm until 2019. Nitrogen is required by pigs in a significant amount, still most of nitrogen in the diet is excreted again via feces and urine. Van der Peet-Schwering et al. (1999) estimated excretions of N, relative to input by feed, varying on average from 38% for weaning pigs, 63% for growing-finishing pigs, and 75% or sows. Nitrogen excretion can by reduced by matching the protein/amino acids contents of the diet as close as possible to the pigs' requirement. Protein levels are generally higher than actually required (Aranink, 2007). Different studies show that protein content of the diet could by reduced by 30-40 g/kg without any effect on growth rate or feed efficiency, when limiting amino acids are supplemented to diets (Lenis and Schutte, 1990; Dourmed et al., 1993; Cahn et al., 1998). According to Rademacher (2000) approximately 25% of the protein in typical corn and soybean diet can not be used, because of unbalanced amino acids. There amino acids are broken down and the nitrogen is excreted as urea in urine.

Therefore, this experiment was conducted to evaluate the effect of dietary crude protein reduction of the growth performance, blood profiles, and nutrient digestibility in weaning pigs.

# II. Review of Literature

## 1. Digestive Physiology of Young Pigs

### 1) Development of digestive enzymes

From 28-36 days' gestation after breeding, the growth rates of the stomach, intestines and pancreas are positively allometric relative to that of whole fetus (Marrable, 1971). Provided that the sow suckles the newborn pig, this pattern of growth continues during the first week after birth. After this time, and while the pig's sole source of feed is the sow's milk, the rate of growth of the stomach, small intestine and pancreas are isometric or negatively allometric relative to that of body weight until the weaning time.

With these size developments, enzyme secretion also changes with age in young pigs. The low acid secretory capacity in stomach at birth and its rapid increase during the first week is probably related to the immaturity of many of the parietal cells in the newborn and the increase in parietal cell size and number which occurs within this period. Because parietal cell which is main tissue to secrete hydrochloric acid (HCl) is not fully developed, and proteolytic activity in the stomach of newborn pigs is very low. Total gastric protease or pepsin activity increases with age because of increased tissue weight and enzyme activity per unit of tissue (Lewis et al., 1957; Hartman et al., 1961). And positive linear relationships between maximal acid secretion and body weight have been reported by Cranwell (1985c) in pigs from birth to 5 - 6 weeks of age.

During the period of early development the pH of stomach remains high, therefore pepsin activity is limited. Early research on the development

of pepsin activity in neonatal pigs suggested low acid secretion was observed until pigs became 2 to 4 weeks of age (Lewis et al., 1957; Hartman et al., 1961). This may be caused by the lack of acid secretion, by the buffering capacity of milk, saliva, gastric mucous and regurgitated bile and pancreatic juice (Maner et al., 1962; Cranwell, 1985). Conversely, evidence of marked acidity in stomach, based only on pH measurements, may have reflected the presence of organic acid, especially lactic acid, produced by gastric fermentation rather than the results of acid secretion (Cranwell et al., 1976). In the studies by Cranwell (1985), which is related to gastric secretion development, sucking pigs which has received no creep-feed were compared with litter mates which were reared by the sow until weaning at 21 days, but were allowed accesses to solid feed (creep-feed) from 14 days and were entirely dependant on solid feed after weaning. The results is that pigs fed solid feed was significantly greater than that of the sow-reared pigs and indicated that the gastric acid secretory capacity developed more rapidly in animals provided with creep feed and weaned on to solid feed. The greater acid secretory capacity was related to them having more stomach tissue per unit body weight and to the stomach tissue itself being capable of producing more acid per unit stomach weight (Cranwell, 1985)

And pancreatic enzyme activities steadily increase from birth to weaning (Pierzynowski et al., 1993b). Cranwell (1995) reported that the reason of increase of pancreatic enzyme activity was due to the relative size if pancreas itself increased. The relative amounts of trypsin remained more or less constant during the first 5 weeks and it is not until 6 weeks of age that there is a substantial increase, due mainly to a significant increase in trypsin activity in pancreatic tissue. In the study by Corring et al. (1978) it coincided with the time that there have been a 260% increase in dry matter intake (solid feed and sow's mild) and a 150% increase in protein intake

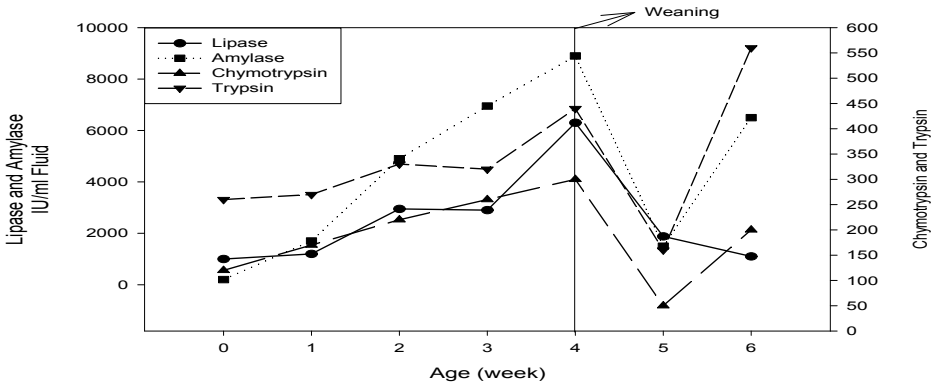
(milk protein and creep-feed protein) at 6 weeks compared to 4 weeks of age. The relative amounts of trypsin were again higher at 8 weeks of age following further increased in dry matter and protein intake (Corring et al., 1978). The development of chymotrypsin follows a somewhat different pattern from that for trypsin. The 1.5- to 3.3- fold increase in the relative amounts of chymotrypsin up to weaning are followed by a dramatic decrease in the week following weaning, and a recovery to amounts similar to or greater than those immediately before weaning in the subsequent 2 - 4 weeks. However, the increase in relative amounts of chymotrypsin 2 - 4 weeks after weaning is due to an increase in the relative size of the pancreas not to a dramatic increase in chymotrypsin activity in pancreatic tissue (Cranwell, 1995). And the size of the relative increase in pancreatic amylase which occurs early in life is in part due to the extremely small amounts of amylase present in the pancreas at birth. Increases in total amounts of amylase over the 6 - 8 week period of the order of 103 - 104 were recorded (Cranwell, 1995). And there was a dramatic decrease in the relative amounts of amylase in the week following weaning, and an equally dramatic recovery to similar or significant greater relative amounts in the 2 - 4 week postweaning period (Corring et al., 1978; Poerzynowski et al., 1993b). Pancreatic lipase also undergoes large relative increase during the first 4 weeks. It is probable that this is not just an effect of age since sow's milk contains a high concentration of lipid (Klobasa et al., 1987), and both the amount and the lipid content of milk increase during the first 3 - 4 weeks of lactation (Elsley, 1970). Also, in the studies by Lindemann et al. (1986), in which pigs were weaned at 4 weeks of age, and Cera et al. (1988), in which pigs were weaned at 3 and 5 weeks of age, predominantly carbohydrate-protein diets, the relative amounts of lipase declined in the 2 weeks after weaning. This is in contrast to the findings in suckling pigs (Corring et al., 1978) in

which lipid intake and relative amounts of pancreatic lipase continued to increase up to 6 weeks of age and were still relatively high at 8 weeks.

Whereas the digestive action of the pancreatic enzymes takes place in the lumen of the intestine, the actions of the various digestive enzymes found in enterocytes takes place at the cell surface or brush border (microvilli), or in the cell itself. The two major groups of digestive enzymes in enterocytes are carbohydrases and peptidases (Alpers, 1987). These enzymes are further complicated because each enzyme has its own characteristic distribution along the length of the small intestine which may change according to the age of the pig and the feeding method (Kidder and Manners, 1980). The lactase activity of small intestine were found to be high at birth and remained its high activity during the first 7 - 10 days of postnatal life (Widdowson et al., 1976). In newborn and one day-old pigs the specific activity of lactase was found to be highest in the proximal region and lowest in the distal region of the small intestine, but at 6 - 10 days of age it appeared to be more evenly distributed along the intestine (Widdowson et al., 1976). However, lactase activity in suckling pigs was observed to undergo an initial marked decrease sometime during the second to fifth week of age which was followed a period when it remained relatively constant or continued to decrease gradually up to 8 weeks of age (Aumaître and Corring, 1978; Kidder and Manners, 1980; Hampson and Kidder, 1986). In the studies by James et al. (1987a) and Sangild et al. (1991a), sucrase and maltase were found to be present at low activities in the small intestine at birth. From 1 week of age the specific activities of both maltase and sucrase increased quite rapidly to reach a peak at 10-16 days and to plateau at about 3 weeks (Aumaître and Corring, 1978; James et al., 1987a; Sangild et al., 1991a). After 3-4 weeks of age sucrase and maltase activities in suckling pigs have been reported to remain at similar plateau levels up to 6-8 weeks or continue to increase up to 8 weeks

of age (Aumaître and Corring, 1978; Kidder and Manners, 1980). At weaning of 3 or 5 weeks of age the specific activities of sucrase, isomaltase and lactase at 5 days after weaning were significantly (two- to five- fold) lower than those in suckling pigs of similar ages (Miller et al., 1986). A reduction in lactase and sucrase specific activities during the 3-8 day period following weaning at 3 weeks of age has also reported by Hampson and Kidder (1986). By 11 days after weaning there was a partial recovery of sucrase activity to the pre-weaning levels, whereas lactase activity continued to decline in both weaned and unweaned pig during this time. In the studies by Hampson and Kidder (1986) and Miller et al. (1986), the reductions in specific activity of brush-border enzymes in the period following weaning were large, and reflected the significant 2.1- to 4.8- fold reductions in maximal enterocyte carbohydrase activities along the length of the villi observed by Miller et al. (1986). In terms of peptidase, in the study of Sangild et al. (1991a), which investigated the development of the specific activities of three brush border peptidase (aminopeptidase A and N, and dipeptidyl peptidase IV) in homogenates from the upper jejunum of newborn and suckling pigs, the overall trend was for peptidase activities to be high at birth or in the first postnatal and decreased with age with specific activities being significantly lower at 34 - 36 days than at 0-7 days of age.

Figure 1. Effect of age on pancreatic enzyme activity(weaning at 4 week)




From above discussion it is evident that the major factors which influence the development of digestive enzymes are age and method of feeding (suckling, creep-feeding or solid feed). Among of them, weaning is considered to be the most critical factor. Lindemann et al. (1986) explained these marked changes of digestive enzymes of young pigs at weaning and is figured above (Figure 1).

## 2) Changes of digestive physiology in weaned pigs

The young sucking piglet has a limited digestive capacity which adequately copes with liquid, sow's milk and water as dietary component. Sow's milk provides piglets with: ⅰ) a controlled, semicontinuous source of highly digestible and available nutrients; ⅱ) a supply of immunological and non-immunological protective agents, and ⅲ) a supply of stimulatory and regulatory factors which may be important for the development of the digestive tract and its regulatory systems. Therefore piglets could not easily adapt to an abrupt change to a solid feed, mainly of plant origin, at weaning. This abrupt dietary change of diets is associated postweaning stress commonly generates digestive disorder such as diarrhea and results in poor

growth performance. Animals suffering postweaning stress showed a reduced feed intake and a shift in the partitioning of dietary nutrients away from skeletal muscle development toward a metabolic response to support the immune system. This may also accelerate lipolysis and muscle protein degradation. In these cases animals may even suffer a net loss in weight rather than increase in weight and growth. There are several explanation for physiological change that young piglets experience with dry feed components. They are described below.

**(1) Enzyme**

In terms of digestive enzyme, firstly, it is widely known that proteolytic enzyme activity in the stomach of newborn pigs is very low. Thus young piglet depends on maternal milk which is composed of lactose. The lactose can be converted to lactic acid by certain microorganism in the stomach, therefore it can help to maintain the low pH of stomach until the hydrochloric acid (HCl) secretion starts. Hydrochloric acid is a limiting factor of activation of proteolytic enzyme, pepsin, in animals. Hydrochloric acid secretion of young pigs could be started by nearly 15 days of age, and its amount is not adequate before 24 days of age (Cranwell et al., 1976), therefore early weaned pigs has suffered form inefficient protein digestion. Moreover, there is a low level of the necessary pancreatic and intestinal enzymes to deal with the new and different feed component as mentioned previously.

**(2) Gut morphology**

Morphological development of the small intestine is very important in terms of digestion and absorption activity. The basic functional unit of the intestine is villus. The villi and lamina propria play an important role in the

digestion and absorption of nutrients. The mucosal epithelium of small intestine is anatomically and functionally immature in neonatal pigs (Gaskins and Kelley, 1995). Marked alteration in the structure of the small intestine was occurred. Hampson (1986) suggested that weaned pigs have highly significant increases in crypt depth and increased complexity of villus morphology with a dramatic reduction in villus height when compared to unweaned pigs. Similary, Cera et al. (1988) demonstrated longer and slender villi in the 2-d-old suckling pig. These long villi also had slightly smaller diameters relative to later postnatal ages. Although villous height declined only slightly from d 2 to 10, the diameter of the villi increased. But these changes of morphology would face the dramatic alteration by weaning time.

And several studies (Kenworthy, 1976; Pluske et al.., 1996b) also reported a reduction in villous height and an increase in crypt depth after weaning. If villous atrophy occurs via an increased rate of cell loss, then this is associated with increased crypt cell production and hence increased crypt depth. According to Hampson (1986), villous height was reduced to 75% of pre-weaning values within 24 hours after weaning and continued to decline until the fifth day after weaning to approximately 50% of the initial values found at weaning. These findings were confirmed by Miller et al. (1986), whereas Cera et al. (1988) observed at weaning time of 3 and 5 weeks of age, a reduction villus height (65%) in the mid-jejunum and a cessation of growth of the small intestine during the 3 days following weaning at 3 weeks of age and also a reduction villus height (27%) was observed although it is not more severe than the pigs weaned at 3 week. When pigs weaned at 3 weeks of age, small intestinal weight and villus height increased in the period from 3 days until 14 and 21 days after weaning, respectively, when they were similar to those in 5-week-old suckling pigs. In the pigs weaned at 5 weeks of age the equivalent recovery in villus height took only 7 days. In

addition to a reduction in villus height, Cera et al. (1988) found that the length of the microvilli (brush border) was reduced in the 3-7 day period following weaning at 3 weeks of age. And the results of the experiments by Kelly et al. (1991 a, b), in which pigs were weaned at 14 days and fed a weaning diet by gastric incubation (force feeding), would suggest that the deleterious changes in small intestinal morphology and carbohydrate enzyme activities which occurred immediately after weaning are in part due to low postweaning feed intakes. These morphological responses to weaning have a great effect on mucosal functions in the small intestine. Several reports clearly observed that villous atrophy and crypt hyperplasia is usually associated with a decline in the activities of the brush border enzymes (Hampson and Kidder, 1986; Miller et al., 1986) Intestinal microflora population was changed at weaning time. Populations of all groups of bacteria declined after weaning. Aerobes, *Lactobacilli* and bacteroides-clostridia most markedly and increased pathogenic bacteria. Because of this reason, weaned piglets frequently have diarrhea. *E. coli* strains are generally considered to be the main cause of diarrhea after weaning of the pigs. Tzipri et al. (1980) indicated that combined infections of rotavirus and certain *E. coli* strains are etiologically important. It is not sufficient explanation to cause diarrhea with only these bacteria because they also exist in the gut of healthy animal. However important thing is that *E. coli* strains become successively predominant in the fecal flora of weaned piglet because weaning piglets are no longer protected by the milk of the sow, an important factor in milk that protects piglets during the suckling period from *E. coli* colonization and undigested feed caused by a shortage of digestive enzyme creates a fertile environment which *E. coli* can proliferate readily (Nabuurs, 1998).

And young pigs should be acquired passive immunity by the ingestion of colostrum and milk with its rich supply of antibodies (Cranwell and

Moughan, 1989). However, passive antibody-mediated immunity, which derived from colostral immunoglobulins, reached a maximum in the pig when 24 to 36 hours old and then decreased logarithmically to precariously low levels when about 3 weeks old (Speer et al., 1959; Miller et al., 1962; Porter, 1976). This time course of passively acquired immunity emphasized the immunological vulnerability of the young pig because the piglet experienced a period when its circulating (humoral) immunity is dangerously low at about 3 weeks of age before significant antibody production occurred by itself (Haye and Kornegay, 1979). Further, it has been reported that a pig's decreased physiological maturity from the stress of weaning at an early age lowered antibody synthesis (Haye and Kornegay, 1979). Gwazdauskas et al. (1978) reported that calves immunized with heterologous erythrocytes within 24 hour of weaning developed fewer erythrocyte agglutinating antibodies than did those not immunized near weaning. A similar reduction in antibody synthesis has been shown in 5-wk-old pigs immunized 24 hour before weaning (Blecha and Kelly, 1981) or 3-wk-old pigs immunized on the day of weaning (Haye and Kornegay, 1979). In the study of Blecha et al. (1983), related to weaning age, weaning pigs when younger than 5-wk-old caused physiological changes detrimental to cellular immune activity, therefore those changes could alter susceptibility in young pigs. Moreover weaning diets have antigenic components which may induce an immune response, especially plant protein source of diets. Wilson et al. (1986) reported that pigs weaned at three weeks old absorbed feed protein antigen from the intestine, and the amount of antigen absorbed declined over the next three weeks. It is associated with an increasing level of serum antibody to the fed protein. In another studies (Li et al., 1990; 1991a; 1991b), immunologically-active soybean protein such as glycinin and β-conglycinin could stimulate a localized immune response which might result in villous

atrophy, crypt hyperplasia and increase anti-soy IgG titers. This boosted immune system could be alleviated while the pig became systematically tolerant to soy protein in some weeks after weaning (Heppell et al., 1989). In the method of pre-weaning diets, further, Newby et al. (1984) hypothesized that a short-term exposure to creep feed and low feed consumption may sensitize the pig to antigen in certain feed ingredients. Second exposure of the sensitized pigs to the dietary antigens at weaning may result in an immune response that damages the lining of the intestinal tract.

## 2. Importance of Protein in Weaning Pigs

### 1) Dietary protein content

Protein is the second most abundant nutrient required in swine diets, ranging from about 26 percent in neonates to 13 percent in adults. The pig does not have a requirement for intact protein, but rather the newborn pig, which is dependent on intact immunoglobulins in sow's colostrum for acquisition of passive immunity. The protein and amino acid reviewed in a few publications (ARC, 1981; NRC, 1998). Although pigs require amino acids for maintenance, growth, and production, they are predominantly fed intact protein to meet their dietary requirements. To formulate and evaluate swine diets, the protein content of the diet or specific feedstuff is commonly calculated based on the nitrogen content. The N content of diets and feedstuff is determined chemically by the Kjeldahl method (Association of Official Analytical Chemists, 1984). The protein content is then calculated by multiplying the N content by 6.25, based on the fact that the average protein contains 16 percent N. This conversion factor invalid for mist feedstuff used in swine diets. However, some protein sources contain non-protein N (NPN), milk

contains several sources of NPN, such as urea, free amino acids, polyamines, and nucleotides.

## 2) Dietary protein source

### (1) Soybean meal proteins

Soybean protein in the form soybean meal has long been the predominant protein source in swine diets. Unfortunately, soybeans contain many anti-nutritional factor such as trypsin inhibitors, lectins, and complex carbohydrates and proteins that impair the pigs' ability to utilize them. Heat treatment of the soybeans in the process of making soybean meal removes much of the trypsin inhibitor. However, complex proteins and carbohydrates are not removed. Complex proteins in soybean meal have been suggested as the cause of a transient hypersensibility response in the early-weaned pig. Before weaning, pigs an consume soybean protein by eating small quantities of sow feed or creep feed and become exposed to the soy proteins. Bourne (1984) explains that prior to building up a tolerance to an antigen such as those in soybean proteins, the pig goes through a period of hightened responsiveness. Feeding the soybean protein during this period can result in damaging hypersensitivity responses, such as increased crypt cell division and the appearance of immature enterocytes on the villous, resulting in reduced digestive and absorptive capacity and an increased susceptibility to enterotoxins. This responses appeared to be caused by antigenic proteins present in soybeans, such as glycinin and beta-conglycinin. The transient hypersensitivity is measured experimentally as higher immunoglobulin G titers to soybean protein resulting fro the pig's attempt to mount an immune response against the antigenic proteins. However, the end result is that digestive abnormalities, including disorders in digestive movement and inflammatory responses in the intestinal mucosa, can occur. Villi are sloughed from

the small intestinal mucosa and absorptive capabilities are reduced.

### (2) Spray-dried animal plasma

Spray-dried animal plasma contains high levels of cysteine, but low methionine levels, making it necessary to formulate for methionine in addition to total sulfur amino acids. When comparing various plasma sources, solubility and bacterial levels should be considered. Higher solubility indicates less heat denaturing during the spray-drying process. Lower bacterial levels are an indication of quality of the raw material. The main response to adding plasma to the diet is an increase in feed intake. Owen et al. (1995) demonstrated that the immunoglobulin fraction of plasma appears to provide the greatest benefit in feed intake, as compared to the albumin fraction or the remaining portions of plasma. In some european countries, animal proteins can not to be fed to pigs due to concerns with Bovine Spongiform Encephalitis (BSE). Thus, in is not legal to use spray-dried plasma in swine diets in these countries. Because of this ban, diets must be formulated using many of the alternative protein sources.

### (3) Dried skim milk

Dried skim milk is being used in weaner diets in some instances because pigs look cleaner and drier when fed a diet containing skim milk. The problem is that they do not grow faster. Pigs fed diets containing dried skim milk often have netter feed efficiency than pigs fed diets containing plasma. However feed intake and average daily gain will be lower. Nevertheless, in addition to being more expensive than other protein sources, our findings indicate that the skim milk can be replaced with lower cost protein sources without sacrificing performances.

## 3) Protein metabolism

The concept of homeorhesis, as define as "the partitioning of nutrients

towards a tissue of priority for a particular physiological state " by Bauman and Currie (1980), is possibly no better illustrated as by the conservation or increase in whole body protein, particularly in the gut, during the weaning-induced fast and subsequent undernutrition. In the case of the newly-weaned pig, the tissue of priority are gut and skeletal muscle protein. Thus, the neonatal pig has an enormous capacity to deposit protein and the whole-body fractional protein synthesis rate is at its highest in the first few weeks of life (Young, 1970). In the face of undernutrition the weaned pig attempts to conserve protein in the gut and to a lesser extent in skeletal muscle tissue. For example, Ebner et al. (1994) found that during periods of both protein and energy reduction, the decrease in protein deposition was less in the gastrointestinal tract than it was in skeletal muscle. Also, calorimetric studies indicate the whole-body protein balance is positive over the first week after weaning despite the animals being in negative energy balance (Bruininx *et al.,*2002). Nevertheless, the newly weaned pig must be in negative protein balance for at least the first 2 days after weaning since they consume so little food over this period of time. Extrapolation of the relationship between protein intake and protein balance suggests that the weaned pig seeds to consume 3.1g protein/$kg^{0.75}$ (Le Dividich *et al.,*1980), or approximately 60g/d of a typical weaner diet, to remain in zero protein balance. Using the slaughter balance technique, Whiitemore et al. (1981) found that newly-weaned pigs were in a slight negative protein balance for the first 4 days after weaning. However, there is no doubt that very soon after commencing to consume solid food the newly-weaned piglet is in positive protein balance and there is a rapid expansion of the gut and other visceral organs. It is also interesting that the rate at which protein deposition increase with increasing feed intake is greater at low as compared to high levels of energy consumption (Close and Stanier, 1984).

Feeding stimulates the fractional rate of protein synthesis and protein accretion in the small intestine and skeletal muscle of young pigs (Davis *et al.,*1996; 1997). While the post-prandial increase in insulin stimulates protein synthesis in

skeletal muscle, insulin does not appear to be the mediator of the increase in intestinal protein synthesis that occurs after feeding (Davis *et al.,*2001). Likewise, systemic elevations of amino acid concentrations, achieved through intravenous infusion, have little effect on intestinal protein synthesis stimulation (Davis et al.2002). Therefore, it appears that the feeding-induced increase lumen rather than via increased arterial supply of insulin or amino acids. In this context, dietary amino acids make a greater contribution to small intestinal protein synthesis than circulation amino acids in fed neonatal pigs (Stoll *et al.,*1999). As the weaned pigs moves from a liquid milk diet to period of limited feed consumption followed by a gradual increased ingestion of a dry complex diet, there are some dramatic changes to intestinal growth and histology. It is likely the some of the hormonal changes that occur postweaning help to conserve gut and body protein.

## 4) Somatotropin and insulin-like growth factor-I

Weaning itself results in a decrease in plasma IGF-I and a simultaneous increase in plasma ST (White *et al.,*1991). The study of Matteri et al. (2000) quite clearly demonstrated that the decrease in IGF-I concentrations was related to the onset of weaning rather than being a developmental response since animals that remained nursing the sow had much higher levels of IGF-I than weaned pigs. Also, the decrease in plasma IGF-I occurs regardless of weaning age, at least between 14 and 35 days (White *et al.,*1991; Matteri *et al.,*2000). Circulation IGF-I does not return to pre-weaning values until 1 to 2 weeks postweaning at a similar time to when pre-weaning energy intakes are achieved (Le Dividich and Seve, 2000). Exogenous IGF-I and analogues inhibit pST secretion in 60kg growing pigs (Dunaiski *et al.,*1997), and it is possible to the increase in pST after weaning is in response to the profound decrease in IGF-I and subsequent diminution in the inhibitory effects of IGF-I upon pST secretion. The increase in pST in response to the reduction in feed intake at weaning may occur in an attempt to conserve gut and

skeletal muscle protein. indeed, the proteins in the gastrointestinal tract have an enormous turnover and without some conservation of these organs the gut would reduce in size to a greater extent than it does immediately after weaning. Interestingly, in weaned pigs fed either a high or low feed intake, the hepatic ST receptor mRNA was down-regulated whereas it was upregulated by a low feed intake in the four muscles examined (longissimus, rhomboideus, soleus, and cardiac) (Katsumata *et al.,*2000). In contrast, at 2 d postweaning Matteri et al. ,(2000) did not see any effect of weaning on either skeletal muscle, adipose tissue or hepatic ST receptor mRNA. However, there were differences between different weight lasses. These adaptations to underfeeding may explain hoy skeletal muscle is spared during the weaning -induced negative energy balance via muscle is spared during and/or elevated circulating pST. Indeed, the effects of pST on protein metabolism differ between the fed state via increasing protein synthesis and decreasing protein degradation and amino acid oxidation (Vann *et al.,*2000a), whereas protein loss is minimized during the fasted state via an increase in protein synthesis and a decrease in amico acid oxidation (Vann *et al.,*2000b).

## 5) Insulin

Insulin plays a key role in the regulation of growth and tissue deposition in the young pig. Insulin stimulates the partitioning of amino acids to protein deposition and away from oxidation and gluconeogenesis while also stimulation glucose incorporation into fat (Dunshea *et al.,*1992). In the suckling pig, insulin increase skeletal muscle protein synthesis although this response declines between 7 and 26 d of age (Davis *et al.,*2001;2002). However, insulin has no effect upon protein synthesis in visceral and intestinal tissues nor is there a decline in protein synthesis over this age range (Davis *et al.,*1996), and although there are little supporting data, it is reasonable to assume that insulin decrease immediately postweaning. For example, plasma insulin decrease precipitiously on the day

following weaning (Rantzer *et al.,*1997) but returns to pre-weaing values within 2-3 days. Similarly plasma insulin was comparavle 5-7 days after weaning as in pigs that had been maintained on liquid cow's milk (Pluske, 1995). Also, plasma insulin was higher in pigs weaned on to a liquid milk replacer diet than in those weaned into a cereal diet on d2 after weaning but not subsequently (McCracken *et al.,*1995). Thus, a decrease in insulin immediately after demands of the young pig. A decrease in insulin would also favour an increase in gluconeogenesis since elevated insulin inhibits gluconeogenesis (Dunshea *et al.,*1992). Presumably, weaning would cause a decrease in protein synthesis in skeletal muscle but not necessarily in visceral and intestinal tissues. This may partially explain how total and relative intestinal masses are maintained, or even increased, during the immediate postweaning period. in addition, re-feeding stimulates protein synthesis in visceral and intestinal tissues via a mechanism independent of insulin and post-hepatic amino aicd supply (Davis *et al.,*2002). The inference is that once pig do commence feeding after weaning then visceral mass will be stimulated, in part because of first-pass utilization of nutrients from the gut lumen (Stoll *et al.,*1999). On the other hand, the increase in skeletal muscle protein synthesis is due to the post-prandial increase in insulin rather than directly to an increase in nutrients (Davis *et al.,*2002).

## 3. Amino Acid Utilization in Weaning Pigs

### 1) Substance of amino acid metabolism

Amino acid metabolism is complex because of the large number of metabolites involved. Amino acid metabolism can be split into those 20 amino acids used for protein biosynthesis. They also function as precursors for the synthesis of many signaling molecules. They are distinct from the unusual amino acids (e.g. ornithine) used for a large variety of intermediary pathways and activated one-carbon units used for the synthesis of aromatic, nitrogen containing compounds

such as nucleic acids and all its cofactor derivatives like nicotinamides and coenzyme A, ubiquinone, heme and chlorophyll.

Because of the multitude of pathways, only a selected few are presented here demonstrating basic principles found in all other metabolic pathways of nitrogenous compounds. Amino acids serve as precursors for lipids, carbohydrates, and nucleic acids including ribonucleotides used as cosubstrates and coenzymes in the production of energy (ATP, NAD, FAD, CoA). Following the metabolic fate of carbon atoms of dietary amino acids, they can be traced to all major metabolic intermediates because of the close interaction of amino acid metabolism with both the citric acid cycle and glycolysis/gluconeogenesis. These intermediates containing carbons from dietary amino acids include pyruvate, acetyl-CoA and acetoacetyl-CoA (ketone bodies), and the citric acid cycle intermediates a-ketoglutarate, succinyl-CoA (heme synthesis), fumarate, malate, and oxaloacetate. Amino acid metabolism is 'separated' into pathways according to the different length of carbon structures involved. These are referred to as the C3, C4, and C5 families of amino acids. which produce common end products during catabolism. The C3 family includes alanine, serine (glycine), and cysteine, all of which are degraded to pyruvate. They are glucogenic amino acids because they can directly be utilized by the liver gluconeogensis (except their amino groups which are excreted as urea). The C4 family of amino acids includes aspartate and asparagine, which are degraded to oxaloacetate and are closely linked to glutamate and alpha-ketoglutarate interconversion by amino transferases. The C4 amino acid threonine has a separate pathway leading to pyruvate and is a glucogenic amino acid. The C5 family of amino acids includes glutamine, proline, arginine, and histidine, all of which are converted ultimately to glutamate, which is deaminated to alpha-ketoglutarate. The non-polar C4 amino acids methionine, isoleucine and valine are precursors for the synthesis of odd numbered fatty acids via the intermediate propionyl-CoA (a C3 acyl-CoA). Propionyl-CoA can be reused for succinyl-CoA (C4) synthesis

(carboxylation) which in turn serves as heme precursor. The non polar amino acid leucine, however, undergoes a more complex degradation pathway, including a decarboxylation-carboxylation detour leading to the formation of acetyl-CoA and acetoacetyl-CoA (a ketone body). Aromatic amino acids include phenylalanine, tyrosine and tryptophan. Phe and Tyr are closely related. They contain a benzene ring which is hydroxylated in tyrosine. Tyrosine is synthesized directly from the essential amino acid phenylalanine. Tryptophan contains a conjugated indole ring and its metabolism is linked to that of vitamin B (niacin C00253). These metabolic relations give rise to an intricate nutritional dependence. For example, a high level of dietary tyrosine relieves the need for essential phenylalanine. Also, metabolic disorders like the impairment of synthesizing tyrosine from phenylalanine makes the former an essential amino acid. This lack of amino acid biosynthetic pathways in humans is the cause of many diseases associated to malnutrition. Pellagra is a vitamin deficiency syndrome caused by an inadequate supply of niacin (vitamin B) because of problems in the pathway leading from tryptophan to niacin synthesis. Vitamin C (C00072), which is a necessary coenzyme in tyrosine metabolism, or vitamin B6 (C00250), which is required for tryptophan metabolism, cause deficiencies in the metabolism of aromatic amino acids. Degradation of aromatic ring structures is mostly performed in liver, also many specialized cells can use the benzene and indole rings for the synthesis of more complex, biologically important molecules such as heme, pigments, and hormones.

Amino acid synthesis includes the fixation of nitrogen in form of ammonia and the assimilation of the latter into keto acids to form amino acids by means of glutamate dehydrogenase and glutamine synthetase. The keto acids are provided by glycolysis and citric acid cycle. In total, there are six anabolic pathways for amino acids referred to as biosynthetic families. Many non essential amino acids can directly be obtained by transamination from glutamate to the respective keto acid (e.g. pyruvate to alanine; oxaloacetate to aspartate). Essential amino acids have to be

obtained from dietary proteins. Plants and micororganisms have all pathways for the net synthesis of amino acids needed for protein biosynthesis. The synthesis of aromatic ring containing amino acids is discussed below.

Table 1. Essential and non-essential amino acids

| Essential amino acids | Non-essential amino acids |
|---|---|
| Histidine | Alanine |
| Isoleucine | Arginine |
| Leucine | Aspartate |
| Lysine | Asparagine |
| Methionine | Cystein |
| Phenylalanine | Glutamate |
| Threonine | Glycine |
| Tryptophan | Proline |
| Valine | Serine |
| | Tyrosine |

Amino acid biosynthesis is under allosteric feed back regulation. In general, the end product of a pathway, the amino acid, inhibits the enzyme catalyzing the first (or committed step) of its own biosynthetic pathway. This ensures the energy saving synthesis of building blocks for protein biosynthesis. Accumulating amino acids thus shuts down their biosynthetic activity. A simple feed back mechanism is found in E.coli. Here, isoleucine, which is derived from threonine, inhibits threonine deaminase (EC 4.2.1.16), the committed step in isoleucine biosynthesis which forms alpha-ketobutyrate or oxo-butanoate. For the biosynthetic pathways of more complex and branched aromatic amino acids, however, an analogous sequential feedback mechanism has been elucidated, as found in the bacteria Bacillus subtilis. Simply put, the endpoint of each branch inhibits the first enzymatic step of the immediately preceding branching point. Thus, the aromatic amino acids do not only inhibit their early common pathways leading

to shikimate, chorismate, or prephenate intermediates. This assures flexibility for the cell by adjusting the levels of aromatic amino acids according to actual needs. Protein synthesis may need different amounts for phenylalanine than tryptophan. Thus tryptophan may shut down its own biosynthetic branch by inhibiting anthranilate synthase, leaving chorismate mutase unaffected. Chorismate will still be used for phenylalanine or tyrosine formation before it accumulates to shut down the entire pathway. Although E.coli uses the same pathways for the synthesis of aromatic amino acids, it uses a different control mechanism to ensure relative independence between the formation of phenylalanine, tyrosine, and tryptophan. Instead of using this simple sequential feedback mechanism after branching points, E.coli relies on enzyme multiplicity meaning that E.coli uses three different enzymes (2-Dehydro-3-deoxyphosphoheptonate aldolase) for the early synthesis of shikimate. Each enzyme is under allosteric control of its 'own' amino acid end product. Thus the level of enzymes in the cytoplasm determine the level of shikimate and chorismate. For phenylalanine and tyrosine, an additional enzyme multiplicity control is used to allosterically suppress two isoforms of chorismate mutase, one of which is specific for Phe, while the other binds only Tyr. Overall, enzyme multiplicity, too, shows a sequential feedback mechanism because of the multiple branching points of the pathway.

## 2) Amino acid utilization for body protein deposition

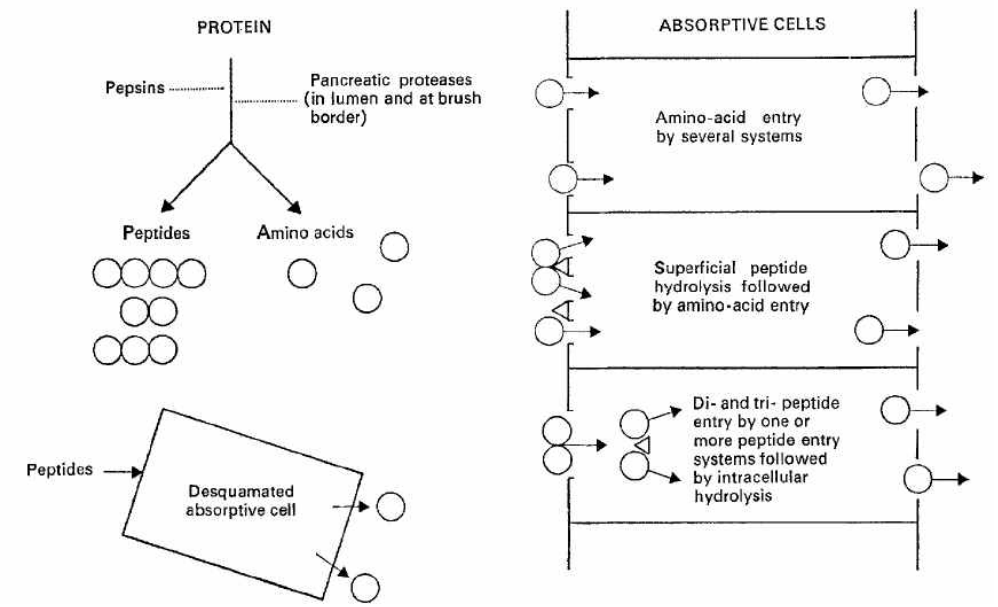
Body proteins are continuously being formed(protein synthesis) and broken down (protein degradation). In an adult animal, synthesis and degradation are equal and body protein is neither gained or lost. However, in a growing animal, synthesis exceeds degradation and this results in protein deposition or accretion. Dietary indispensable amino acid are used for a variety of metabolic processes in the body and are precursors for a wide range of biologically active compounds, but it is protein deposition that accounts for the greatest amino acids use. The efficiency with

which absorbed amino acids are used for protein deposition depends on several factors including genetic differences and whether the amino acids intake is limiting or in excess of the requirement. In growing-finishing pigs consuming diets that are limiting in protein, the most limiting acid will be used more efficiency than other amino acids, and it is the efficiency of utilization of this amino acid that will affect hoy well the overall dietary protein is used.

## 3) Absorbtion

In general, only free amino acids are entering the portal blood during protein absorption, protein digestion products leave the intestinal lumen in the forms 1) as free amino acids and 2) as small peptides. Amino acids are absorbed by carrier-mediated transport, and for glycine and most L-amino acids, transport has been shown to be active. In adult small intestine, energy transport is derived mainly from oxidative metabolism (De la Noue, 1970). The active transport mechanisms appear to be situated in the plamsa membrane of the mucosal poles of the absorptive cells, since amino acids are concentrated in the cells while transport is going on. The transport mechanisms were stereochemically specific for L-amino acids, but it has been shown that several D-amino acids are actively transported ( De la noue, 1970). The active transport of amino acids by mucosal cell is, like that of many other solutes, sodium dependent. The transport of sodium and amino acids is coupled by linkage to common carriers. Sodium is pumped out of the cells by the sodium extrusion system that is found in all animal cells and is linked direct to metabolic energy. The maintain a low concentration of sodium within cells. Sodium enters the cells down the resulting gradient via the coupled transport systems, bringing amino acids with it and enhancing them to be concentrated intracellularly.

Figure 2. Tentative scheme of protein absorption (Matthews, 1972)




The intestinal absorbtion of amino acid is affected by the quality and quantity of dietary protein intake. The time course is longer and the quantity of amino acid absorbtion greater when peptides are infused compared to free amino acids. Although pigs, like other species, appear to have the apability to absorb small peptides intact into the mucosa(Matthews, 1991), there is very limited evidence of the appearance of peptides, of dietary origin, in the portal blood. Intestinal amino acid absorbtion is more efficient and quantitatively greater when pigs ingest purified protein supplements, such as casein and rapeseed meal, than protein in cereal grains, like wheat, barley, and corn. However absorbtion of wheat protein is more rapid than that of corn or barley. The net absorbtion of amino acids increases with protein intake, although differences exist for specific amino acids. Moreover, the amino acid absorbtion coefficient are consistently lower than the ileal digestibility estimates. Therefore, intense rate of metabolism of luminal amino acids by the intestinal mucosal enterocytes during absorbtion.

## 4) Nitrogen balance

The maintenance protein requirement is defined as the intake necessary to maintain N balance. The daily maintenance requirement for protein represents the obligatory n loss associated with urinary and endogenous fecal N measured in pigs fed a protein-free diet. Urinary N losses represent catabolized amino acids and other nitrogenous components, whereas fecal N represents undigested dietary protein and endogenous secretions together with proteins and nucleic acids produced by colonic microbial fermentation.

Amino acid catabolism represents a source of inefficiency in protein utilization. Amino acids can serve as gluconeogenic precursors in the pigs. Thus, limiting energy intake results in inefficient use of dietary protein because amino acids are used for synthesis of glucose rather than tissue protein. The suppression of amino acids catabolism and urea synthesis and in response to dietary energy appears to result from increased circulating insulin concentrations. The metabolic effects of dietary-energy ratio on protein utilization become important when a pig's energy intake varies. Because pigs tend to eat to satisfy their energy needs, total feed intake may decrease when the dietary energy content increase due to reduce energy content when fiber is added to the diet. To circumvent the problems associated with energy intake on protein utilization, protein and amino acid requirements have been expressed per unit of digestible energy. Another potential source of inefficiency in the utilization of dietary amino acids is the metabolism and endogenous excretion of amino acids by the small intestine. The rate of amino acid appearance in portal circulation consistently underestimates the disappearance rate of dietary amino acids from the intestinal lumen(Stoll et al., 1998). Thus, pattern of amino acids appearing in the portal circulation is the indicator of protein quality and utilization. On the other hands, the loss of essential amino acids at the terminal ileum represents a true metabolic inefficiency, because these amino acids are catabolized by microbial fermentation. As dietary protein intake increases, the contribution of endogenous

amino acids to the ileal output decreases curvilinearly, even though the true ileal amino acid digestibility stay content, whereas the efficiency of amino acid absorbtion tends to decrease with increasing amino acid intake. About 75 to 80 percent of the endogenous proteins secreted into the lumen are digested and reabsorbed in the small intestine (Souffrant et al., 1993). Endogenous amino acid output at the terminal ileum in pigs tends to be relatively higher in threonine and sulfur amino acids than is reflected in protein consumed in the diet or deposited in the body. As a result, maintenance requirement for growth (Hahn et al., 1995). The ileal amino acid composition also tends to be enriched in the nonessential amino acids.

## 4. Reduction of Nitrogen Excretion in Weaning Pigs

### 1) Legislation of nitrogen emission

As many swine farms have expanded and become more specialized in these days, heavy environmental load becomes main problem to them. So the disposal of animal manure becomes a challenge in the intensive production of swine because the amount and nutrient composition of the manure may exceed the capacity of the land to accept it as a fertilizer. Major environmental load is divided into mineral load to the soil and gaseous load to the air. Manure excretion of swine farm caused mineral overloaded and especially surplus nitrogen flow into ground and surface waters, causing high nitrate levels in ground water. To solve the problems, many countries established many legislation for controlling nitrogen excretion and reducing environmental pollution. They regulate the effluent water from livestock manure by area of pig house considering effluent water quality. Government of Japan limited the upper emission level by 80-160 mg/L of BOD, 80-160mg/L COD, 120-200 mg/L of SS, 16 mg/L of total phosphorus and 120 mg/L of total nitrogen. Also, they suggested the daily emission guideline for 120 mg/L of BOD and COD,

150 mg/L of SS, 8 mg/L of total phosphorus and 60 mg/L of total nitrogen, respectively. European union (EU) have been issued to regulate environmental pollution by many directives from different countries. Moreover, every country of EU has its own legislation for controlling environmental pollution, but these should comply with the EU council. They had set three directive environmental legislation: 1) integrated pollution prevention and control directive (IPPC Directive 96/61/EC); 2) nitrate directive (Directive 91/676/EEC); 3) national emission ceilings directive (NEC Directive 2001/81/EC). The aim of the IPPC directive is to reduce emissions to air, water and soil, and to make efficient use of resources. Also, this directive is focusing on large farms (>2,000 growing-finishing pigs; >750 sows; >40,000 chickens). These farm should use the best available techniques (BAT) to reduce environmental pollution. EU suggested nitrate directive aims to prevent pollution of water by excess nutrients or nitrogen from agriculture. In EU countries, nitrate levels in ground water are set to a maximum of 50 mg/L and the yearly N-application with manure should not exceed 170 kg/ha. In case of Japan, they establish the legislation of manure emission and water pollution

Korea government presented the plan about standard of effluent water from manure purification plant. According to ministry of agriculture food and rural affairs, they suggested the emission legislation that total nitrogen content will be reduced until 2019 gradually (850mg/L, 2012 year → 500mg/L, 2016 year → 250mg/L, 2019 year). In 2012, Korea government revised legislation of effluent water from manure purification plant strictly. Before the revision, purification plant emitted effluent water under 850 ppm of total nitrogen, but they cannot emit the effluent water from manure above 250 ppm of total nitrogen after the revision.

## 2) Nutrition and nitrogen excretion

Ammonia in pig manure mainly derived from the breakdown of urea in

urine. On the other hand, only a few ammonia comes from the breakdown of protein in the feces (Aarnink et al., 1993). The converting rate of urea to ammonia depends on the urease activity and urine mixed with faeces or solid floor that urease is present. For this reason, major ammonia emitted from manure pit in a clean pig pen (Aarnink et al., 1997). Furthermore, ammonia from the manure is a slow process governed by factors as ammonia concentration, pH, temperature, air velocity and emitting surface area. In figure 3, the emission of ammonia in the nitrogen chain of growing-finishing pigs is suggested. This figure demonstrated that almost half of the nitrogen excreted by urine and faeces can emit during storage of the manure inside the pig house and during surface application of the manure.

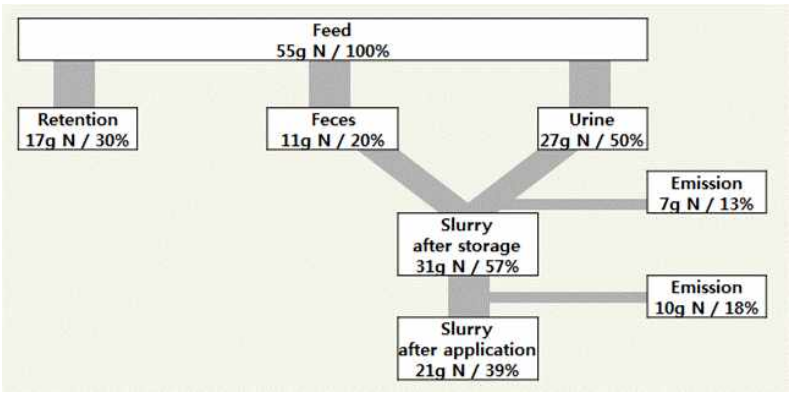



Figure 3. Nitrogen chain in growing-finishing pigs (Aarnink, 1997)

Furthermore, excretions of nitrogen, relative to input by feed, estimated varying on average from 38% for weaning pigs, 63% for growing-finishing pigs and 76% for sows (Van der Peet -Schwering et al., 1990). Similarly, weaning pigs had 47% N output/N intake and growing-finishing pigs had 67% N output/N intake (Table 2).

Table 2. Amounts of nitrogen output for different classes of swine (Dourmad et al., 1992

| Class | Nitrogen output | | | |
|---|---|---|---|---|
| | Per pig (g/d) | Per space (kg/year) | % of total | N-output /N-intake (%) |
| Sows[1] | | | | |
| Replacement gilts | 51 | 186 | 1.7 | 69 |
| Weaned sows | 42 | 103 | 0.9 | 73 |
| Gestation | 40 | 954 | 8.7 | 77 |
| Lactation | 79 | 459 | 4.2 | 57 |
| Piglets | | | | |
| Suckling (27d) | 1 | 54 | 0.5 | 14 |
| postweaning (to 25kg) | 11 | 907 | 8.2 | 47 |
| Growing-finishing pigs (25 to 105 kg) | 38 | 8,360 | 75.8 | 67 |
| Total | | 11,023 | 100.0 | 65 |

[1]Calculated for a 100-sow equivalent to 89 productive sows

Kornegay and Harper (1997) also reported that only 20-50 % of the nitrogen consumed is retained in the body (Table 3). In other word, 50-80 % of the nitrogen consumed is excreted into the environment. This means the possibility for using dietary strategies to reduce nitrogen excretion.

Table 3. Digestion and retention of nitrogen by different classes of swine (Kornegay and Harper, 1997).

| Item | Nursery | Finishing | Gestating | Lactating |
|---|---|---|---|---|
| Nitrogen, % | | | | |
| Digestion | 75-88 | 75-88 | 88 | - |
| Retention | 40-50 | 30-50 | 35-45 | 20-40 |

The urea excretion through the urine can be reduced by improving the nitrogen utilization within feed. Many researchers conducted experiments for achieving the aim. Aarnink et al. (1993) demonstrated that reduction of dietary crude protein 10 g/kg reduced ammonium nitrogen content in manure 9%. Also, Sutton et al. (1998) reported that ammonium nitrogen and total nitrogen concentrations decreased by lower dietary crude protein content from 130 to 100 g/kg with limiting amino acids supplemented. Canh et al. (1998) suggested that lower protein content in the diet with 165, 145 and 125 g/kg reduced ammonia emission. In these results, reduction of crude protein content of the diet, averaging 10 g/kg, had a 10% lower ammonium content of the manure and a 10 - 12.5% lower ammonia emission. In similar, Latimier and Dourmad (1993) reported that the value of relative reduction in nitrogen excretion and ammonia emission. In other research, Kay and Lee (1997) presented that reductions in ammonia emission of 58% in growing pigs and of 46% in finishing pigs when the crude protein content of the diets were reduced by 60 - 65 g/kg, respectively. Kerr (1995) demonstrated a conclusion of 28 experiments that for each 1 experiment reduction in crude protein, nitrogen excretion was reduced by 8.4 percent. According to Latimier (1993), feeding low protein diets (grower 16.2% and finisher 13.5%) reduce the nitrogen emission in the air of approximately 15 percent compared to control diets (grower 17.8% and finisher 15.4%), respectively. In addition, nitrogen excretion was reduced by 9% and ammonia emission by 8.6% per 1% reduction in crude protein. Hobbs et

al. (1996) conducted the experiment about a comparison between diets high in protein (20.8% crude protein for the grower and 18.9% crude protein for the finisher) and diets low in protein (16.1% crude protein for the grower and 13.8% crude protein for the finisher). In the result of this experiment, they observed a reduction in odor compounds when pigs were fed diets low in protein.

# Ⅲ. Effect of dietary crude protein reduction on growth performance, blood profiles, and nutrient digestibility in weaning pigs


## Abstract

This experiment was conducted to evaluate the effect of dietary crude protein reduction on growth performance, blood profiles, and nutrient digestibility in weaning pigs. A total of 128 crossbred piglets ([Yorkshire × Landrace] × Duroc), weaned at 21 ± 3 d of age with average initial body weight of 7.68 ± 0.2 kg were used in this experiment during 5 weeks. Weaning pigs were assigned to one of 4 treatments in a randomized complete block (RCB) design in 8 replicates with 4 pigs per pen. Control diet had same level of dietary crude protein as NRC (1998) recommended, and 2% of dietary crude protein was reduced from 2% to 6% less then NRC recommendation. Three phase feeding programs were used in this experiment (phase I, d 0 to 7; phase II, d 7 to 21; phase III, d 21 to 35). In growth performance, reduction of dietary crude protein resulted in decrease of growth performance, but 2% reduction of dietary crude protein treatment showed significantly same rows with control diet ($P$<0.05). Pigs fed control diet showed the highest serum blood urea nitrogen (BUN) concentration, but insulin-like growth factor-1 (IGF-1) level was decreased by dietary crude protein reduction (linear, $P$<0.05). Sixteen crossbred barrows (4 pigs per treatment) averaging 16.28 ± 1.82 kg body weight were alloted in apparent nutrient digestibility. Dry matter digestibility was increased by dietary crude protein reduction (linear, $P$<0.05; quadratic $P$<0.01), and 4% reduction of dietary crude protein treatment showed significantly higher dry matter

digestibility than other treatment ($P$<0.01). Four percent reduction of dietary crude protein treatment showed significantly power total nitrogen excretion than other treatment ($P$<0.01). Also, dietary crude protein digestibility was lowered by dietary crude protein digestibility (linear, $P$<0.05), and crude ash digestibility was increased by dietary crude protein reduction (linear, $P$<0.05; quadratic, $P$<0.05). Crude fat digestibility showed some tendency of increased than decreased, and 4% reduction of dietary crude protein treatment showed the highest crude fat digestibility (quadratic, $P$=0.08). In nitrogen retention, fecal nitrogen was decreased by dietary crude protein reduction (linear, $P$<0.05), and also, urinal nitrogen showed decreased tendency by dietary crude protein reduction ($P$=0.067). Consequently, reduction of dietary crude protein level in weaning pig diet reduce growth performance, but improve nutrient digestibility. And 4% dietary crude protein reduction could be acceptable for commercial weaning pigs feed with considering impact of environmental pollution.

**Key Words** : Crude protein, Nitrogen excretion, Weaning pig, Growth performance, Diarrhea

# Introduction

Weaning is one of the most critical periods in modern pig production cycle. Stresses in response to simultaneous stressors such as mixed with other pigs, moved to different environment, and fed grain-based diet rather than sow's milk cause post weaning growth lag and diarrhea. To optimize performance of piglets at weaning phase, in-feed antibiotics have been used as growth promotor and the therapeutic treatment of gastrointestinal diseases (Verstegen and Williams, 2002). After banning of antibiotics use in animal feed, Dermot and Helen (2003) demonstrated that ban of antibiotics in animal feed made problems about postweaning diarrhea until the finishing stage. And increase of use of antibiotics for medication was observed in Denmark. The increase of postweaning diarrhea due to the banning of antibiotics use in animal feed has stimulated the use alternative managements and nutritional strategies. In nutritional solutions, undigested feed could increase the fermentation in large intestine and cause occurrence of diarrhea. And reducing dietary crude protein level has been reported to limit the frequency and severity of digestive problems in weaning pigs. The content of dietary CP, individual AA, or both may affect the formation and concentrations of metabolites resulting from microbial fermentation (Hobbs et al., 1996). And high dietary CP concentration, as is common in diets for early-weaned pigs, may increase microbial fermentation of undigested protein, and encourage proliferation of pathogenic bacteria in the gastrointestinal tract (Ball and Aherne, 1987). So, reducing protein levels in weaning pig diet could reduce the post weaning diarrhea and economical loss. Consequently, the objective of current study was to evaluate the effects of reducing crude protein level in the diets on growth performance, blood profiles, and nutrient digestibility in weaning pigs.

# Materials and Methods

***Animals, experimental design and housing***

A total of 128 crossbred piglets ([Yorkshire × Landrace] × Duroc), weaned at 21 ± 3 d of age with average initial body weight of 7.68 ± 0.2 kg were obtained from a single source (The Jacob farm, Umseong, Korea) and transported for approximately 1 hour to the Seoul National University experimental farm (Suwon). The experimental animals were subjected to 4 treatments experiments. The treatments were: 1) Control (basal diet, NRC requirement), 2) P2 (reduce 2% CP levels from basal diet), 3) P4 (reduce 4% CP levels from basal diet), 4) P6 (reduce 6% CP levels from basal diet). Piglets were alloted to 4 treatments by body weight and sex in a randomized complete block (RCB) design with 8 replicates 4 pigs per pen.

All pigs were housed in a plastic woven floored pen, equipped with a feeder and two nipple waterer and allowed *ad libitum* access to feed and water throughout the whole experimental period. The temperature was maintained at 32 ℃ in the first week and decreased 1 ℃ every week, and 27 ℃ in the last week. Body weight and feed intake were recorded weekly to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain per feed ratio (G:F ratio). Diarrhea scores were recorded twice a day (8 AM, 8 PM) with the range from 1 (healthy) to 5 (severe) throughout the whole feeding trial period.

***Experimental diet***

The feeding trial was divided into three phases (phase I, d 0 to 7; phase II, d 7 to 21; phase III, d 21 to 35). In phase I, diet were formulated to contain 1.35% lysine, 0.44% methionine, 0.80% calcium and 0.65% phosphorus, in phase II, diet were formulated to contain 1.15% lysine, 0.37% methionine, 0.75% calcium and 0.63% phosphorus and phase III, diet were

formulated to contain 1.05% lysine, 0.34% methionine, 0.70% calcium and 0.60% phosphorus (NRC, 1998). The basal diet contained approximately 3,265 kcal of ME in all phases (d 0 to 35). All other nutrient factors were met or exceeded NRC (1998) recommendation. The formula and chemical composition of experimental diet in all phases were presented in Table 1, 2, 3.

***Diarrhea occurrence***

Diarrhea occurrence was checked for entire feeding trial twice every day. Each pig showed watery diarrhea in each pen considered 1 point, and average number of pigs showed diarrhea in pen was calculated.

***Blood profiles***

Blood samples were collected from anterior vena cava of the same pigs weekly during the whole experimental period for blood urea nitrogen (BUN), creatinine, insulin-like growth factor-1 (IGF-1) analysis. Collected blood samples were quickly centrifuged for 15 min at 3,000 rpm and 4 ℃. The serum were carefully removed to plastic vials and stored at -20 ℃ until BUN, creatinine, IGF-1 analysis. Total BUN concentration was analyzed by using blood analyzer (ADIVA1650, Bayer, Japan), and IGF-1 concentration in blood was measured by using hormone analyzer (Immulite 2000, DPC, SUA)

***Apparent nutrient digestibility***

Digestive trial was conducted to evaluate the nutrient digestibility and nitrogen retention. A total 16 crossbred barrows (4 pigs per treatment) averaging 16.28 ± 1.82 kg body weight were individually allotted to metabolic crates. After a 5 days adaptation period, pigs were involved in a 5 day collection period. Total collection method was used to investigate nutrient

digestibility. A 1.5g of chromium oxide was added in experimental diet (Phase II diet of feeding trial) of the first and the last day of collection period for selection marker. During the experimental period, water was provided *ad libitum* and all pigs were fed 150g twice a day. Excreta and urine were collected everyday and stored -20 ℃ until analysis. Collected excreta were dried in an air-forced drying oven at 60 ℃ for 72 h, and ground into 1 mm particles in a Wiley mill for chemical analysis including moisture, protein, fat, ash, Ca, P contents. Total urine was collected daily in a plastic container containing 50 ml of 4N $H_2SO_4$ and frozen during the 5 day collection period for nitrogen retention analyses.

***Ileal amino acid digestibility***

A total of 12 crossbred barrows averaging 6.54 ± 0.34 kg body weights were surgically fitted with T-cannula at the distal ileum, approximately 7 cm cranial to the ileo-cecal sphincter. After surgery, pigs were allotted in an individual pen (slatted floors made of plastic, 0.93 × 1.53 $m^2$) for 2 week recuperation. After recuperation period, pigs were subjected to 4 days of adjustment period and thereafter 3 days of collection was done. A 0.5% of chromium oxide was supplemented as a marker with each phase II diets of feeding trial, and protein free diet was formulated for standardized apparent ileal amino acid digestibility. One hundred thirty grams of experimental feed were provided twice a day (7:00 AM, 7:00 PM), and ileal digesta was collected using polyester bag during 10 hours (from 9:00 AM to 7:00 PM). Water was provided *ad libitum*. Polyester bags were removed every hour and digesta were collected in plastic bottle, and frozened at -60°C

until later analysis. After collection period, samples were dried with freeze drier (Inshin, Freeze drier, TFD series) during 48 hours. Then, freeze dried ileal digesta were ground to fine powders and hydrolysis with 6.0N HCl. Constituent amino acid in experimental feed and ileal digesta were measured with AAA(Amino Acid Analyzer, Waters 2690). The AID of AA was calculated with chromium contents in the diets and digesta by the indirect method. Ileal endogenous AA losses, induced by the N-free diet, were used for calculating SID. The calculations of AID and SID of AA were conducted according to Stein et al.(2007) as shown below:

$$AID\ (\%) = [1 - (AA_{ie} / AA_{d}) \times (Cr_{d} / Cr_{ie})] \times 100$$

$$BEAL\ (g/kg\ of\ DMI) = AA_{nie} \times (Cr_{nd} / Cr_{nie})$$

$$SID\ (\%) = AID + [(BEAL / AA_{d}) \times 100]$$

where, $AA_d$ and $Cr_d$ = ratio of AA and Cr in the diet

$AA_{ie}$ and $Cr_{ie}$ = ratio of AA and Cr in the ileal digesta

$Cr_{nd}$ = ratio of Cr in the N-free diet (g/kg of DM)

$AA_{nie}$ and $Cr_{nie}$ = ratio of AA and Cr in the ileal digesta of N-free diet (g/kg of DM)

BEAL = Basal endogenous AA losses

***Chemical analysis***

Analysis of the experimental diets and excreta was conducted according to the methods of the AOAC (1995). Kjeltec 2000 (Foss) was used for crude protein analysis.

***Statistical analysis***

The means for ADG, ADFI, G/F, and blood assay were analyzed as

a randomized complete block (RCB) design using the General Linear Model (GLM) procedure of SAS. Experimental pen was used as an experimental unit for the performance data, whereas individual pig data served as the experimental unit in nutrient digestibility and standardized ileal amino acid digestibility. All of collected data for performance and digestibility were carried out by comparing means according to least significant difference (LSD) multiple range tests, using the General Linear Model procedure of SAS (SAS Institute, 2006).

# Results and Discussion

***Growth performance and diarrhea occurrence***

Effect of restricted dietary crude protein levels on growth performance were shown in table 8. In body weight, quadratic effect was observed in the first week (quadratic, $P<0.05$). And in the 5th week, body weight was decreased as dietary protein reduced (linear, $P<0.01$), and CON and P2 treatments showed significantly higher body weight than P4 or P6 treatment ($P<0.01$). During phase II, P6 treatment showed lower ADG than other treatments ($P<0.05$), and linear effect was observed ($P<0.01$). Gain:Feed ratio was decreased by dietary reduction (linear, $P<0.01$). In phase III and overall experimental period, similar with phase II, ADG and G:F ratio were decreased by dietary protein reduction (linear, $P<0.01$), and CON and P2 treatments showed higher growth performance than P4 and P6 treatments ($P<0.01$). Htoo et al. (2007) reported that supplementation of high protein diet increased growth performance numerically, but significant difference was not observed. And in the study of Nyachoti et al. (2006), ADG, ADFI and G:F ratio were decreased by dietary protein reduction. Also, other previous studies reported that decrease of feed intake of low protein diet, and both of decreased ADG and ADFI resulted in maintenance of G:F ratio (Southern and Baker, 1982; Le Bellego and Noblet, 2002). However, in current study, feed intake was not affected by dietary crude protein reduction significantly, and only showed some tendency during phase III ($P=0.073$). Consequently, reduction of dietary crude protein resulted in decrease of growth performance, but 2% reduction of dietary crude protein than NRC (1998) recommendation could be acceptable in weaning pig diet without detrimental effects on growth performance.

Ball and Aherne (1987) reported that feeding high-protein diets

increased the incidences of diarrhea in swine. However, Le Bellego and Noblet (2002) revealed that effect of low protein diet was not observed in weaning pig diarrhea. In current study, diarrhea occurrence was checked entire experimental period but only 4 pigs showed diarrhea among the all experimental pigs during first week, and no pigs showed diarrhea in phase II and phase III.

***Blood profiles***

The effect of reduction of dietary crude protein on BUN, serum creatinine and serum IGF-1 concentrations were shown in Figures 4, 5 and 6. The BUN concentration have related to protein quality in diet, and have negative relation with amino acid balance in body (Eggum, 1970). So, BUN concentration have been used as index for determine the protein requirement (Cai et al., 1996) and requirement of single amino acid (Taylor et al., 1982; Coma et al., 1995) in body. In the result of this experiment, pigs fed control diet showed significantly higher BUN concentration than other treatments in the 1st week, and linear effect of crude protein reduction was observed ($P$<0.05). Although there were no significant differences among treatments but this trend was maintained until the end of experiment. This result was in agreement with Nyachoti et al. (2006) which reported that decrease of dietary protein caused linearly decrease of PUN. These results demonstrated that protein in control diet had more excess amino acids contents than other treatments for utilized in body. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body. In the result of this experiment, serum creatinine concentration were not affected by reduction of dietary protein. IGF-1 is secreted by stimulation of growth hormones, and it is related to growth and differentiation of tissue. IGF-1 affected by nutritional status of animal. IGF-1 supply energy for cell

growth, and have critical rolls in regulation of structure and function of cardiovascular system and born growth (Bayes-Genis, 2000). In the result, linear effects were observed in all periods (linear, $P<0.05$ in 1st and 5th week, $P<0.01$ in 3rd week). However, P6 treatment showed significantly lower serum IGF-1 concentration than CON and P2 treatments in 1st week ($P<0.01$), and showed similar tendency in 3rd week ($P=0.078$). In 5th week, P6 treatment showed significantly lower serum IGF-1 concentration than CON treatment ($P<0.05$). These result indicated that protein reduction caused reduction of serum IGF-1 concentration, and reduction of dietary protein over 4% could be detrimental for growth.

***Digestive trials***

The effect of reduction of dietary crude protein on apparent nutrient digestibility and nitrogen retention were shown in table 9. Dry matter digestibility was increased by dietary protein reduction (linear, $P<0.05$; quadratic $P<0.01$), and P4 treatment showed significantly higher dry matter digestibility than other treatment ($P<0.01$). Crude protein digestibility was lowered by dietary protein level (linear, $P<0.05$), and crude ash digestibility was increased by dietary protein reduction (linear, $P<0.05$; quadratic, $P<0.05$). Crude fat digestibility showed some tendency of increased than decreased, and P4 treatment showed the highest crude fat digestibility (quadratic, $P=0.08$). In nitrogen retention, fecal nitrogen was decreased by dietary crude protein reduction (linear, $P<0.05$). And also, urinal nitrogen showed decreased tendency by dietary crude protein reduction ($P=0.067$). P4 treatment showed lower total N excression than other treatment ($P<0.01$). Result of current study is in agreement with the study of Deng et al. (2007) which demonstrated that decrease of dietary crude protein affect decreasing of total nitrogen loss, fecal nitrogen, urinary nitrogen. They also reported that

decrease of nitrogen retention by dietary crude protein reduction. However, in this experiment. although nitrogen output was increased, but nitrogen retention was increased as dietary crude protein level increased ($P$<0.01). These results indicated that high protein diet have lower digestibility but excess dietary protein for nitrogen retention.

The effect of reduction of dietary crude protein on standardized ileal amino acid digestibility was shown in Table 10. Threonine, Glycine, Valine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Arginine, Proline digestibility were decreased by dietary protein reduction (linear, $P$<0.05), and P6 treatment showed significantly lower Threonine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Arginine, Cysteine digestibility than other treatment ($P$<0.05). In this experiment although amino acid digestibility were decreased, as content of amino acid were decreased. This result is consistent with the result of apparent nutrient digestibility for crude protein level were shown in Table 8. Amino acid digestibility of CON and P2 treatment shows significantly equal level, 2% reduction of dietary crude protein than NRC (1998) recommendation could be acceptable in weaning pig diet without detrimental effects on ileal amino acid digestibility.

# Conclusion

reduction of dietary crude protein level in weaning pig diet reduced growth performance and ileal amino acid digestibility but improved nutrient digestibility. Although high protein diet have had high level of serum blood urea nitrogen concentration, low nutrient digestibility and high nitrogen losses, but growth performance, nitrogen retention and serum IGF-1 concentration were higher in high protein diet than low protein diet. Consequently, 4% reduction could be acceptable for commercial swine feed with considering impact of environmental pollution.

**Table 4. Formula and chemical composition of experimental diets, Phase I**

| Item | Dietary crude protein, % | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| **Ingredients (%)** | | | | |
| EP corn | 21.99 | 26.75 | 31.51 | 36.04 |
| SBM-44 | 34.09 | 30.26 | 26.47 | 22.68 |
| HP300 | 5.15 | 3.59 | 2.00 | 0.39 |
| Whey powder | 3.21 | 3.21 | 3.21 | 3.21 |
| Lactose | 12.00 | 12.00 | 12.00 | 12.00 |
| Barley | 20.56 | 20.92 | 21.27 | 21.91 |
| MCP | 1.08 | 1.18 | 1.28 | 1.36 |
| Limestone | 0.93 | 0.90 | 0.87 | 0.83 |
| L-lysine | 0.20 | 0.38 | 0.55 | 0.72 |
| DL-met | 0.13 | 0.15 | 0.18 | 0.20 |
| Vit. Mix | 0.12 | 0.12 | 0.12 | 0.12 |
| Min. Mix | 0.12 | 0.12 | 0.12 | 0.12 |
| Salt | 0.10 | 0.10 | 0.10 | 0.10 |
| Choline-Cl(25%) | 0.10 | 0.10 | 0.10 | 0.10 |
| ZnO | 0.10 | 0.10 | 0.10 | 0.10 |
| Antibiotics | 0.12 | 0.12 | 0.12 | 0.12 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| **Chemical composition**[a] | | | | |
| ME, kcal/kg | 3265.02 | 3265.01 | 3265.00 | 3265.02 |
| CP, % | 23.00 | 21.00 | 19.00 | 17.00 |
| Lysine, % | 1.35 | 1.35 | 1.35 | 1.35 |
| Methionine, % | 0.44 | 0.44 | 0.44 | 0.44 |
| Ca, % | 0.80 | 0.80 | 0.80 | 0.80 |
| Total P, % | 0.65 | 0.65 | 0.65 | 0.65 |

[a] Calculated value.

**Table 5. Formula and chemical composition of experimental diets, Phase II**

| Item | Dietary crude protein, % | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| **Ingredients (%)** | | | | |
| EP corn | 31.22 | 36.66 | 42.04 | 47.46 |
| SBM-44 | 33.85 | 28.40 | 22.95 | 17.39 |
| HP300 | 0.75 | 0.57 | 0.39 | 0.27 |
| Lactose | 8.00 | 8.00 | 8.00 | 8.00 |
| Barley | 22.69 | 22.72 | 22.81 | 22.98 |
| Soy oil | 0.75 | 0.63 | 0.52 | 0.37 |
| MCP | 1.08 | 1.19 | 1.30 | 1.39 |
| Limestone | 0.80 | 0.77 | 0.74 | 0.69 |
| L-lysine | 0.13 | 0.30 | 0.47 | 0.64 |
| DL-met | 0.07 | 0.10 | 0.12 | 0.15 |
| Vit. Mix | 0.12 | 0.12 | 0.12 | 0.12 |
| Min. Mix | 0.12 | 0.12 | 0.12 | 0.12 |
| Salt | 0.10 | 0.10 | 0.10 | 0.10 |
| Choline-Cl(25%) | 0.10 | 0.10 | 0.10 | 0.10 |
| ZnO | 0.10 | 0.10 | 0.10 | 0.10 |
| Antibiotics | 0.12 | 0.12 | 0.12 | 0.12 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| **Chemical compositions**[a] | | | | |
| ME, kcal/kg | 3265.01 | 3265.00 | 3265.02 | 3265.02 |
| CP, % | 21.00 | 19.00 | 17.00 | 15.00 |
| Lysine, % | 1.15 | 1.15 | 1.15 | 1.15 |
| Methionine, % | 0.37 | 0.37 | 0.37 | 0.37 |
| Ca, % | 0.75 | 0.75 | 0.75 | 0.75 |
| Total P, % | 0.63 | 0.63 | 0.63 | 0.63 |

[a] Calculated value.

**Table 6. Formula and chemical composition of experimental diets, Phase III**

| Item | Dietary crude protein, % | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| **Ingredients (%)** | | | | |
| EP corn | 41.47 | 47.24 | 53.06 | 58.96 |
| SBM-44 | 27.72 | 22.18 | 16.64 | 11.10 |
| HP300 | 0.28 | 0.21 | 0.15 | 0.09 |
| Lactose | 2.00 | 2.00 | 2.00 | 2.00 |
| Barley | 24.77 | 24.48 | 24.14 | 23.72 |
| Soy oil | 1.19 | 1.04 | 0.89 | 0.73 |
| MCP | 0.98 | 1.10 | 1.22 | 1.34 |
| Limestone | 0.69 | 0.65 | 0.61 | 0.57 |
| L-lysine | 0.18 | 0.35 | 0.52 | 0.69 |
| DL-met | 0.06 | 0.09 | 0.11 | 0.14 |
| Vit. Mix | 0.12 | 0.12 | 0.12 | 0.12 |
| Min. Mix | 0.12 | 0.12 | 0.12 | 0.12 |
| Salt | 0.10 | 0.10 | 0.10 | 0.10 |
| Choline-Cl(25%) | 0.10 | 0.10 | 0.10 | 0.10 |
| ZnO | 0.10 | 0.10 | 0.10 | 0.10 |
| Antibiotics | 0.12 | 0.12 | 0.12 | 0.12 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| **Chemical compositions**[a] | | | | |
| ME, kcal/kg | 3265.00 | 3265.02 | 3265.02 | 3265.01 |
| CP, % | 19.00 | 17.00 | 15.00 | 13.00 |
| Lysine, % | 1.05 | 1.05 | 1.05 | 1.05 |
| Methionine, % | 0.34 | 0.34 | 0.34 | 0.34 |
| Ca, % | 0.70 | 0.70 | 0.70 | 0.70 |
| Total P, % | 0.60 | 0.60 | 0.60 | 0.60 |

[a] Calculated value.

**Table 7. Formula of experimental diets in standardized ileal amino acid digestibility**

| Item | Dietary crude protein, % | | | | N-free diet |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| EP corn | 31.05 | 36.46 | 41.81 | 47.21 | - |
| SBM-44 | 33.68 | 28.26 | 22.84 | 17.30 | - |
| Corn starch | - | - | - | - | 70.46 |
| HP300 | 0.75 | 0.57 | 0.39 | 0.27 | 0.00 |
| Lactose | 7.96 | 7.96 | 7.96 | 7.96 | 7.96 |
| Dextrose | - | - | - | - | 15.00 |
| Barley | 22.58 | 22.61 | 22.70 | 22.87 | - |
| Soy oil | 0.75 | 0.63 | 0.52 | 0.37 | 2.20 |
| MCP | 1.07 | 1.18 | 1.29 | 1.38 | 2.64 |
| Limestone | 0.80 | 0.77 | 0.74 | 0.69 | 0.70 |
| L-lysine | 0.13 | 0.30 | 0.47 | 0.64 | - |
| DL-met | 0.07 | 0.10 | 0.12 | 0.15 | - |
| Vit. Mix | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 |
| Min. Mix | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 |
| Salt | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Choline-Cl (25%) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| ZnO | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Antibiotics | 0.12 | 0.12 | 0.12 | 0.12 | - |
| Chromium oxide | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

**Table 8. Effect of dietary protein reduction on growth performance in weaning pigs[1)]**

| Criteria | Dietary crude protein, % | | | | SEM[2)] | P-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | | Lin- | Quad- |
| **Body weight, kg** | | | | | | | |
| Initial | 7.68 | 7.67 | 7.68 | 7.68 | | | |
| 1 week | 8.81 | 8.61 | 8.75 | 8.95 | 0.193 | 0.204 | 0.042 |
| 3 week | 13.72 | 13.69 | 13.28 | 13.19 | 0.253 | 0.096 | 0.913 |
| 5 week | $20.45^{A}$ | $20.04^{A}$ | $18.22^{B}$ | $17.67^{B}$ | 0.371 | 0.001 | 0.876 |
| **ADG, g** | | | | | | | |
| 0-1 week | 162 | 134 | 153 | 182 | 6.693 | 0.201 | 0.042 |
| 1-3 week | $350^{a}$ | $363^{a}$ | $323^{a}$ | $303^{b}$ | 7.751 | 0.009 | 0.259 |
| 0-3 week | 288 | 287 | 267 | 263 | 5.789 | 0.087 | 0.907 |
| 3-5 week | $481^{A}$ | $454^{A}$ | $353^{B}$ | $320^{B}$ | 15.753 | 0.000 | 0.900 |
| 0-5 week | $365^{A}$ | $353^{A}$ | $301^{B}$ | $286^{B}$ | 8.195 | 0.000 | 0.883 |
| **ADFI, g** | | | | | | | |
| 0-1 week | 234 | 225 | 237 | 258 | 5.914 | 0.111 | 0.189 |
| 1-3 week | 621 | 606 | 619 | 607 | 9.796 | 0.746 | 0.937 |
| 0-3 week | 433 | 422 | 432 | 426 | 6.817 | 0.849 | 0.870 |
| 3-5 week | 669 | 601 | 607 | 613 | 10.115 | 0.074 | 0.066 |
| 0-5 week | 528 | 494 | 502 | 501 | 6.603 | 0.217 | 0.209 |
| **G/F** | | | | | | | |
| 0-1 week | 0.692 | 0.587 | 0.648 | 0.710 | 0.021 | 0.470 | 0.037 |
| 1-3 week | $0.564^{AB}$ | $0.599^{A}$ | $0.525^{BC}$ | $0.498^{C}$ | 0.010 | 0.001 | 0.079 |
| 0-3 week | 0.663 | 0.677 | 0.620 | 0.616 | 0.009 | 0.037 | 0.658 |
| 3-5 week | $0.721^{A}$ | $0.759^{A}$ | $0.584^{B}$ | $0.521^{B}$ | 0.024 | 0.000 | 0.163 |
| 0-5 week | $0.692^{A}$ | $0.717^{A}$ | $0.602^{B}$ | $0.569^{B}$ | 0.015 | 0.000 | 0.234 |

[1)] A total 128 crossbred pigs with an average initial body weight 7.68 ± 0.2 kg

[2)] Standard errors of means

[ABC] Mean within rows with different superscripts differ, $P<0.01$

[ab] Mean within rows with different superscripts differ, $P<0.05$

**Table 9. Effect of dietary protein reduction on apparent nutrient digestibility and nitrogen retention in weaning pigs[1)]**

| Criteria | Dietary crude protein, % | | | | SEM[2)] | P-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | | Lin- | Quad- |
| **Apparent nutrient digestibility, %** | | | | | | | |
| DM | $91.54^{B}$ | $91.95^{B}$ | $93.61^{A}$ | $92.14^{A}$ | 0.236 | 0.014 | 0.005 |
| Crude protein | $92.70^{a}$ | $91.97^{ab}$ | $92.97^{a}$ | $90.84^{b}$ | 0.300 | 0.030 | 0.114 |
| Crude ash | $60.91^{b}$ | $64.97^{ab}$ | $70.20^{a}$ | $65.34^{ab}$ | 1.084 | 0.033 | 0.024 |
| Crude fat | $76.43^{B}$ | $71.33^{C}$ | $84.57^{A}$ | $74.01^{AB}$ | 1.412 | 0.359 | 0.080 |
| **Nitrogen retention, g/day`** | | | | | | | |
| N-intake | 9.81 | 8.71 | 7.83 | 7.00 | 1.346 | | |
| N-Feces | $0.72^{a}$ | $0.70^{a}$ | $0.55^{b}$ | $0.64^{ab}$ | 0.116 | 0.021 | 0.105 |
| N-urine | 2.82 | 2.45 | 2.14 | 2.15 | 0.659 | 0.066 | 0.466 |
| N-excretion[3)] | $3.54^{A}$ | $3.15^{AB}$ | $2.69^{B}$ | $2.79^{AB}$ | 0.137 | 0.039 | 0.358 |
| N-retention[4)] | $6.27^{A}$ | $5.56^{AB}$ | $5.14^{B}$ | $4.21^{C}$ | 1.100 | 0.000 | 0.682 |

[1)] A total 16 crossbred pigs with an average initial body weight 16.28 ± 1.82 kg

[2)] Standard errors of means

[3)] N excretion (g) = Fecal N (g) + Urinary N (g)

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

[ABC] Mean within rows with different superscripts differ, $P<0.01$

[ab] Mean within rows with different superscripts differ, $P<0.05$

**Table 10. Effect of dietary protein reduction on apparent ileal amino acid digestibility and standardized ileal amino acid digestibility[1)]**

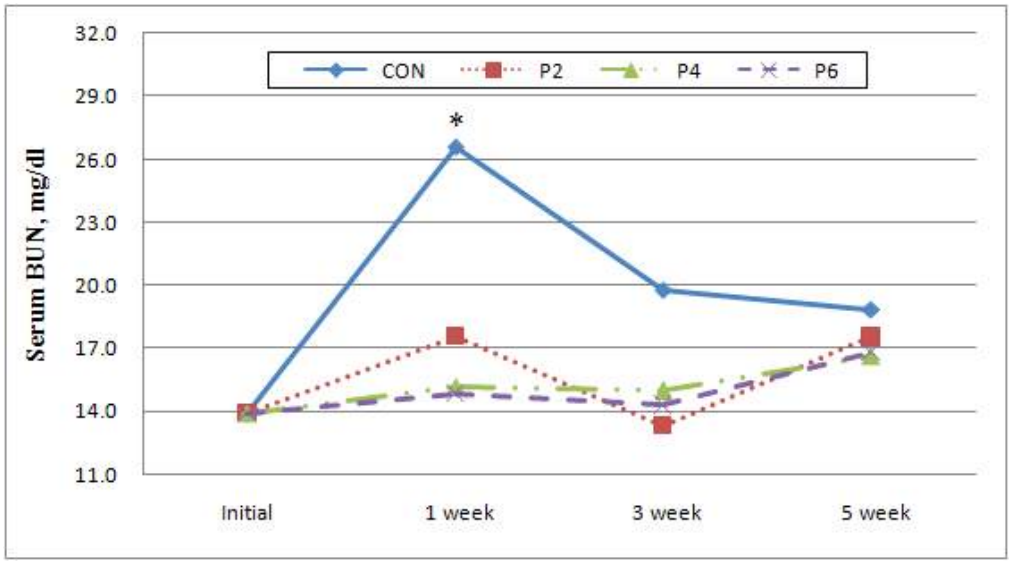
| Criteria | Dietary crude protein, % | | | | SEM[2)] | P-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | | Lin- | Quad- |
| **Apparent ileal digestibility, %** | | | | | | | |
| Aspartic acid | 89.50 | 86.29 | 90.33 | 89.56 | 0.89 | 0.62 | 0.53 |
| Threonine | 83.82$^{a}$ | 83.01$^{a}$ | 81.07$^{ab}$ | 75.94$^{b}$ | 1.24 | 0.01 | 1.21 |
| Serine | 88.08 | 88.37 | 88.05 | 85.99 | 0.63 | 0.12 | 0.20 |
| Glutamic acid | 91.63 | 92.95 | 92.06 | 90.16 | 0.48 | 0.07 | 0.02 |
| Glycine | 84.81 | 84.19 | 82.12 | 80.36 | 1.08 | 0.04 | 0.68 |
| Alanine | 85.47 | 85.14 | 86.87 | 83.14$^{a}$ | 0.93 | 0.49 | 0.33 |
| Valine | 85.96 | 85.72 | 83.92 | 79.67 | 1.07 | 0.03 | 0.27 |
| Isoleucine | 91.25$^{a}$ | 91.69$^{a}$ | 90.62$^{a}$ | 87.37$^{b}$ | 0.67 | 0.01 | 0.05 |
| Leucine | 91.18$^{a}$ | 91.68$^{a}$ | 90.62$^{a}$ | 87.54$^{b}$ | 0.65 | 0.02 | 0.07 |
| Tyrosine | 92.09$^{a}$ | 91.65$^{a}$ | 90.65$^{a}$ | 87.83$^{b}$ | 0.62 | 0.00 | 0.06 |
| Phelylalanine | 91.70$^{a}$ | 92.37$^{a}$ | 91.05$^{a}$ | 88.21$^{b}$ | 0.63 | 0.02 | 0.07 |
| Lysine | 90.12 | 90.53 | 90.97 | 89.57 | 0.49 | 0.78 | 0.36 |
| Histidine | 90.66$^{a}$ | 90.25$^{a}$ | 88.83$^{a}$ | 84.52$^{b}$ | 0.87 | 0.00 | 0.07 |
| Arginine | 94.11$^{A}$ | 93.69$^{A}$ | 92.90$^{A}$ | 90.37$^{B}$ | 0.54 | 0.00 | 0.09 |
| Proline | 88.49$^{a}$ | 88.20$^{a}$ | 87.10$^{ab}$ | 84.40$^{a}$ | 0.79 | 0.01 | 0.20 |
| Methionine | 94.75 | 94.61 | 95.00 | 94.00 | 0.34 | 0.62 | 0.61 |
| Cysteine | 85.49$^{a}$ | 83.83$^{a}$ | 80.83$^{ab}$ | 78.41$^{a}$ | 1.09 | 0.01 | 0.79 |
| **Standardized ileal digestibility, %** | | | | | | | |
| Aspartic acid | 98.62 | 93.89 | 97.65 | 97.72 | 0.90 | 0.90 | 0.23 |
| Threonine | 85.78$^{a}$ | 84.71$^{a}$ | 82.68$^{ab}$ | 77.78$^{b}$ | 1.24 | 0.01 | 0.26 |
| Serine | 94.08 | 93.44 | 93.23 | 91.64 | 0.61 | 0.12 | 0.63 |
| Glutamic acid | 105.77 | 104.55 | 103.89 | 103.30 | 0.47 | 0.08 | 0.72 |
| Glycine | 88.31 | 87.36 | 85.56 | 83.87 | 0.99 | 0.03 | 0.76 |
| Alanine | 88.67 | 88.12 | 89.46 | 86.23 | 0.87 | 0.41 | 0.41 |
| Valine | 89.18 | 88.47 | 86.58 | 82.72 | 1.06 | 0.03 | 0.37 |
| Isoleucine | 92.83$^{a}$ | 92.93$^{a}$ | 91.79$^{a}$ | 88.77$^{b}$ | 0.66 | 0.01 | 0.08 |
| Leucine | 95.75$^{a}$ | 95.35$^{a}$ | 94.25$^{ab}$ | 92.04$^{b}$ | 0.60 | 0.02 | 0.31 |
| Tyrosine | 93.67$^{a}$ | 93.04$^{a}$ | 91.94$^{a}$ | 89.34$^{b}$ | 0.62 | 0.01 | 0.16 |
| Phelylalanine | 93.99$^{a}$ | 94.14$^{a}$ | 92.81$^{ab}$ | 90.31$^{b}$ | 0.61 | 0.01 | 0.14 |
| Lysine | 93.82 | 93.88 | 94.02 | 93.25 | 0.46 | 0.71 | 0.66 |
| Histidine | 91.98$^{a}$ | 91.41$^{a}$ | 89.97$^{a}$ | 85.95$^{b}$ | 0.85 | 0.01 | 0.10 |
| Arginine | 96.50$^{A}$ | 95.81$^{A}$ | 94.86$^{A}$ | 92.69$^{B}$ | 0.53 | 0.01 | 0.21 |
| Proline | 91.34 | 90.94 | 89.96 | 87.59 | 0.73 | 0.02 | 0.30 |
| Methionine | 95.52 | 95.34 | 95.65 | 94.82 | 0.32 | 0.61 | 0.67 |
| Cysteine | 86.61$^{a}$ | 84.91$^{a}$ | 81.92$^{ab}$ | 79.57$^{b}$ | 1.08 | 0.01 | 0.82 |

[1)] A total 12 crossbred pigs with an average initial body weight 6.54 ± 0.34 kg

[2)] Standard errors of means

[AB] Mean within rows with different superscripts differ, $P<0.01$

[ab] Mean within rows with different superscripts differ, $P<0.05$




* Linear effect of crude protein reduction ($P$<0.05)

**Figure 4. Effect of dietary protein reduction on serum blood urea nitrogen (BUN) concentration in weaning pigs**

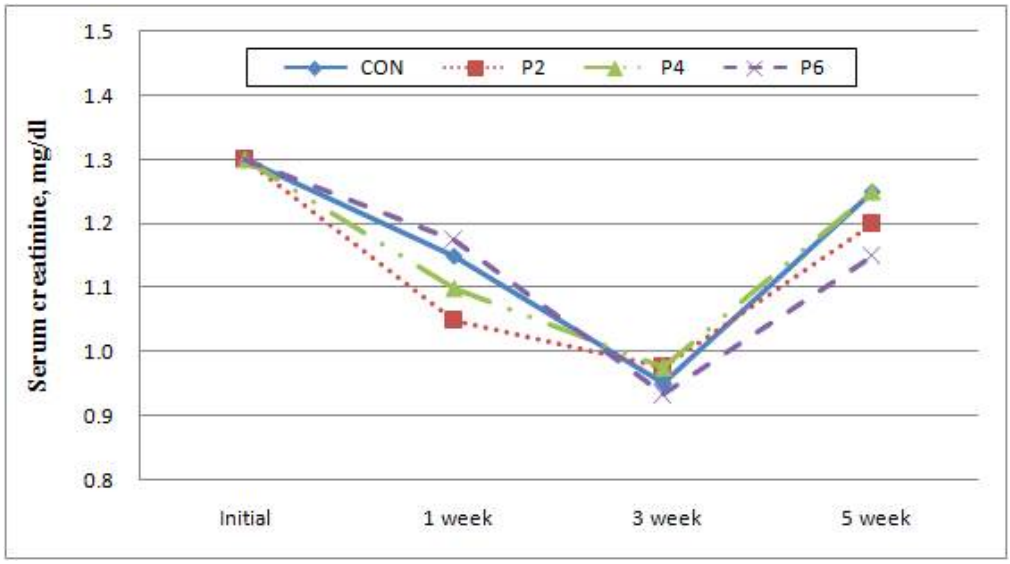
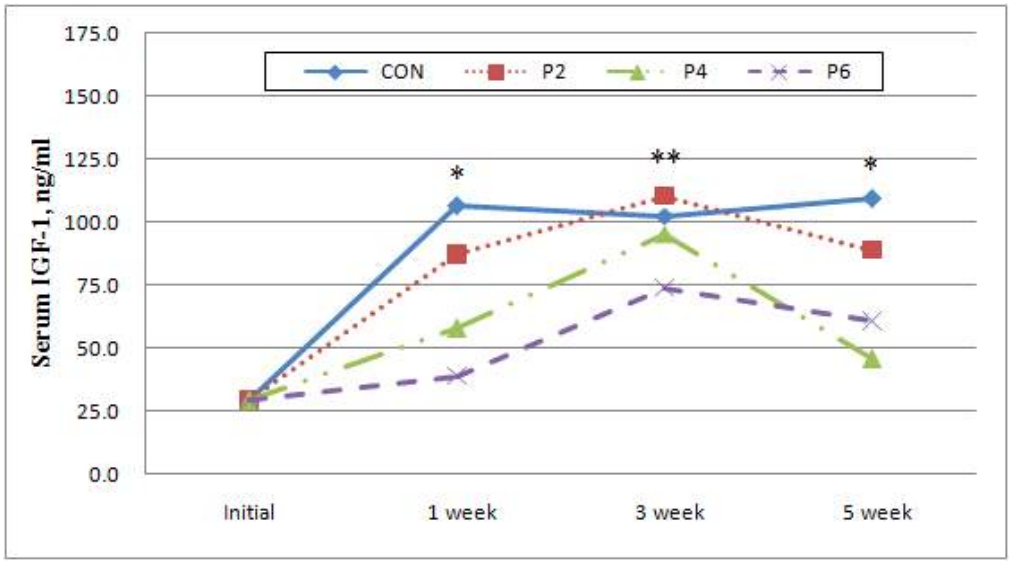



**Figure 5. Effect of dietary protein reduction on serum creatinine concentration in weaning pigs**




* Linear effect of crude protein reduction ($P$<0.05)

** Linear effect of crude protein reduction ($P$<0.01)

**Figure 6. Effect of dietary protein reduction on serum insulin like growth factor-1 (IGF-1) concentration in weaning pigs**

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# Ⅴ. Summary in Korean

이유 후 발생되는 설사와 성장정체를 막기 위한 영양적인 조절방안 중 하나는 사료 내 단백질 함량의 조절이라 할 수 있다. 우리나라의 경우, 이유 후 높은 폐사율과 빠른 성장에 대한 막연한 기대감으로 사료 내 조단백질 함량을 NRC (1998) 요구량 대비 높게 배합해오고 있다. 과잉의 사료 단백질은 소화가 다 되지 못하고 대장으로 유입되어 발효가 되고 이는 설사의 원인이 된다. 또한 조단백질 함량이 높은 원료 사료들은 상대적으로 가격이 비싸 원료사료의 대부분을 수입에 의존하는 국내 형편을 고려하면 매우 비경제적이라 할 수 있다. 더불어 2012년 제정된 축산 폐수 내 총 질소 함량의 규제가 강화됨에 따라, 폐수 내 질소의 주된 요인인 사료 내 조단백질 수준은 더 이상 돼지의 성장 능력만이 아닌 환경을 고려해야 하는 상황이 되었다. 따라서 본 연구는 사료 내 조단백질 함량 수준이 이유자돈의 성장성적, 영양소 소화율, 설사빈도 및 혈액성상에 미치는 영향에 대해 알아보고자 실험이 수행되었다. 21 ± 3일령에 이유한 평균체중 7.68 ± 0.2 kg의 삼원교잡종 ([Yorkshire× Landrace]×Duroc) 128두를 이용하여 사양실험이 진행되었다. 처리구는 CON (NRC 권장량), P2 (조단백질 함량 2% 감소), P4 (조단백질 함량 4% 감소), P6 (조단백질 함량 6%감소)으로 총 4처리였으며 성별 및 체중을 고려하여 돈방 당 4두씩 8반복으로 진행하였고, 각 돈방은 난괴법 (RCBD)에 의해 배치하였다. 사료와 물은 무감소 급여 (*ad libitum*)를 하였고 사료의 변경은 성장 단계에 맞춰 3개의 phase로 단계별 급여하였으며 매 단계마다 체중을 측정하고 경정맥에서 혈액을 채취하였다. 사양성적에 있어서 각 처리구 간 사료섭취량의 차이는 발견되지 않았으며, 사료 내 단백질 함량이 감소이 일당사료섭취량과 사료효율을 감소시키는 것으로 나타났지만 (linear, $P<0.05$), 사료 내 단백질 함량을 2% 수준까지 감소하였을 경우 CON 대조구와 유의적으로 동등한 수준의 성장능력을 보였다 ($P<0.05$). 설사빈도에 있어서는, 실험 전기간 동안 설사를 보인 실험돈이 거의 발견되지 않아 처리구간의

차이가 나타나지 않았다. 혈액성상 분석결과, 대조구가 1주차에 유의적으로 높은 BUN 농도를 보였으며 ($P$<0.05), 조단백질 함량에 따라 BUN이 낮아지는 결과를 보였으며 (linear $P$<0.05), 비록 유의차는 나타나지 않았으나 전 실험기간에 걸쳐 대조구가 높은 BUN 농도를 보였다. 혈중 크레아티닌 농도에는 각 처리구간의 차이가 발견되지 않았다. 하지만 혈중 IGF-1 농도에서는 전 기간에 걸쳐 단백질 함량의 감소에 따라 IGF가 낮아지는 linear effect가 발견되었다 ($P$<0.05). 평균체중 16.28±1.82 kg의 자돈 16마리를 이용하여(4처리 4반복, 완전임의 배치법) 전분 채취법으로 영양소 소화율을 분석하였다. 건물 소화율과 단백질 소화율 경우 사료 내 단백질 함량의 감소에 따라 소화율이 높아지는 결과를 보였고 (linear, $P$<0.05), 모든 영양소에 대해 P4 처리구가 가장 높은 소화율을 나타냈다. 질소 축적율을 계산한 결과 단백질 함량이 높을수록 분으로 배출되는 질소함량과 ($P$<0.05) 뇨를 통해 배출되는 질소함량이 ($P$=0.067) 모두 높아지는 것으로 나타났으나, 그럼에도 불구하고 사료 내 단백질 함량이 높을수록 질소 축적율이 높게 나타났다 ($P$<0.05). 결론적으로 사료 내 조단백질 함량의 감소은 사양성적과 회장 내 아미노산 소화율을 감소시키는 것으로 나타났으며, 영양소 소화율을 증가시키는 것으로 추정된다. 하지만 이유자돈의 성장능력과 환경적 요인을 고려할 때 사료 내 단백질 함량을 4% 수준에서 감소하는 것이 적절한 것으로 사료된다.