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A Dissertation

for the Degree of Doctor of Philosophy

Effects of Energy Modulation and Non Starch Polysaccharide Enzyme on Physiological Responses, Reproductive Performance and Nutrient Digestibility of Sows

모돈 사료 내 에너지 함량의 조절과 NSP 분해효소제의 첨가유무가 임신돈 및 포유돈의 생리적인 변화, 번식 성적 및 영양소 소화율에 미치는 영향

February, 2014

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Effects of Energy Modulation and Non Starch Polysaccharide Enzyme on Physiological Responses, Reproductive Performance and Nutrient Digestibility of Sows

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이 논문을 농학박사 학위논문으로 제출함
2014 년 2월

서울대학교 대학원 농생명공학부
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윤민성의 농학박사 학위논문을 인준함
2014 년 2월

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Overall Summary

Effects of Energy Modulation and Non Starch Polysaccharide Enzyme on Physiological Responses, Reproductive Performance and Nutrient Digestibility of Sows

The objectives of these experiments were 1) to investigate the effect of energy modulation and NSP(Non starch polysaccharide) enzyme supplementation in gestation diet on physiological responses and reproductive performance of sows, 2) to investigate the effect of energy modulation and NSP enzyme supplementation in lactation diet on physiological responses and reproductive performance of lactating sows, and 3) to investigate the effect of dietary energy modulation and NSP enzyme supplementation on nutrient digestibility of gestating sows.

Experiment I. Effects of Energy Modulation and NSP Enzyme Supplementation in Gestation Diet on Physiological Responses and Reproductive Performance of Sows

This experiment was conducted to investigate the effects of dietary energy levels and enzyme supplementation in gestation diet on physiological responses and reproductive performance of sows. A total of 37 multiparous sows (F1, Yorkshire x Landrace, 4.9±1.6 parity ; Darby, Korea) with an initial BW of 216.9 ± 19.6 kg were alloted to one of four treatments with a 2 x 2 factorial arrangement. The first factor was dietary energy levels (3,165 or 3,265 kcal of ME/kg), and the second factor was a NSP enzyme complex (α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea). The experimental diets containing different energy levels and with or without supplementation of 0.1% NSP enzyme were supplied in gestation period according to each treatment. All other nutrients were met or exceeded the requirements of NRC (1998), and sows were fed 2.4 kg of diet daily during gestation. During lactation, the same diet was provided *ad libitum*

regardless of dietary treatments during gestation with a free access to water. During the whole experimental period, there were no significant differences in the results of body weight, backfat thickness, and ADFI in lactation. Even though the significant effects were not detected in body weight, backfat thickness and ADFI during lactation, sows fed diets containing NSP enzyme showed numerically reduced body weight change(0-21 d). In the results of reproductive performance and litter growth, the average weight of piglets at birth ware improved when sows were fed high energy (energy response, $P<0.01$) and enzyme diets (enzyme response, $P<0.03$), and sows fed diet containing 3,265 kcal of ME/kg with 0.1% NSP enzyme showed numerical decrease of litter size without significant difference. Increased plasma concentrations of progesterone at mating and insulin at 70 d were observed when NSP enzyme was added to gestation diet (enzyme response, $P<0.05$), and decreased plasma concentration of glucose at 110 d was observed when dietary energy level increased (energy response, $P<0.05$). The colostrum and milk compositions during lactation including milk fat, protein, total solid and solids-not-fat were not affected by dietary treatments, and the content of lactose at 7 d of lactation was increased when NSP enzyme was added to gestation diet (enzyme response, $P<0.05$). Consequently, feeding diet containing 0.10% NSP enzymes regardless of energy levels showed benefit effects on body weight and back fat thickness change of sows, and the effects of supplementing NSP enzyme was increased as dietary energy level decreased.

Experiment II. Effects of Energy Modulation and NSP Enzyme Supplementation in Lactation Diet on Physiological Responses and Reproductive Performance of Lactating Sows

A study was conducted to evaluate the effect of dietary energy levels and NSP enzyme supplementation in lactation diet on physiological response and reproductive performance of lactating sows. A total of 40 mixed-parity sows (F1, Yorkshire x Landrace, 5.8±0.8 parity; Darby, Korea) with an initial BW of 249.66 ±

8.86 kg were used for a trial, and were allotted to one of four treatments based on BW and backfat thickness in a completely randomized design (CRD) with a 2 x 2 factorial arrangement after farrowing. The first factor was energy level in diets (3,165 or 3,265 kcal of ME/kg), and the second factor was NSP enzyme complex inclusion (α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea). The experimental diets were formulated based on corn and soybean meal and contained 18.98 or 18.88% crude protein, 0.99% lysine, 0.75% Ca, and 0.64% total P, and all other nutrients were met or exceeded the requirements of NRC (1998). During lactation, experimental diets were provided *ad libitum* regardless of dietary treatments with a free access to water. The body weight change of sows was not affected by dietary treatments and numerical reduction of body weight loss from 0 to 21 d of lactation was observed in sows fed diet contained 3,265 kcal of ME/kg compared with those fed diet contained 3,165 kcal of ME/kg in lactation (P=0.09). Interaction effect between dietary energy level and enzyme supplementation was observed in the result of backfat thickness at 21 d postpartum (interaction effect, P<0.05), and WEI of sows was not differed among treatments after weaning. However, ADFI in lactation was decreased when sows were fed high energy (energy response, P<0.01) and enzyme diets (enzyme response, P<0.01). Feeding diets containing 3,265 ME kcal/kg or 0.10% NSP enzyme had no effects on litter size and litter performance of lactating sows except for the average weight gain of piglets from 0 to 21 d of lactation (energy response, P<0.05), and the contents of fat, protein, total solid and solids-not-fat in colostrum and milk also were not changed by dietary treatments. The content of milk lactose at 21 d of lactation tended to be increased when sows were fed diet containing 3,265 kcal of ME/kg compared with those fed diet containing 3,165 kcal of ME/kg (P=0.09). Consequently, when sows were fed low energy diet (3,165 kcal of ME/kg) with dietary NSP enzyme, they showed similar physiological responses and litter performance of lactating sows compared to those fed high energy diet (3,265 kcal of ME/kg).

Experiment III. Effects of Energy Modulation and NSP Enzyme Supplementation on Nutrient Digestibility of Gestating Sows

The experiment was conducted to investigate the effect of dietary energy levels and enzyme supplementation on nutrient digestibility of gestating sows. Four multiparous sows (Yorkshire × Landrace) were allotted in a repeated 4 × 4 Latin-square design with a 2 x 2 factorial arrangements. Four treatments were arranged with 2 main factors of energy levels (3,165 or 3,265 kcal of ME/kg) or enzyme supplementation (0 or 0.1% of NSP enzyme; α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea). After 5 days of adaptation, the excreta were collected for 4 days to analyze the digestibilities of dry matter, protein, ash and fat. There was no significant difference in the results of body weight and backfat thickness during the whole collection period, but numerically improved changes of backfat thickness were detected when sows were fed diet containing 3,165 kcal of ME/kg with 0.10% NSP enzyme compared with those fed low energy diet without NSP enzyme. When sows were fed high energy diets, improved digestibilities of ash and fat were observed relative to those fed low energy diets (energy response, $P<0.01$), and the interaction effect between dietary energy level and supplementation level of NSP enzyme was observed in the digestibilities of dry matter and ash (interaction effect, $P=0.07$ for dry matter and $P<0.05$ for ash), resulted in higher effects of supplementing NSP enzyme in sows fed low energy diet. This study demonstrated that supplementing NSP enzyme contributed a positive effect on digestibilities of dry matter, crude fiber and ash when sows were fed low energy diet (3,165 ME kcal/kg).

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List of Abbreviation

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

Chapter I. General Introduction

The global market for feed enzymes has been rapidly expended during last two decades. According to a research report from Austrian Federal Environment Agency, approximately 57 enzymes are widely used in feed industry. Market of feed enzymes can be broadly divided into phytase (60%) and nonphytase (40%) enzyme. The size of global feed enzyme market is expected to reach $727 million in 2015 from around $344 million in 2007 (Li et al., 2012). Adeola and Cowieson (2011) estimated that $550 million dollar of feed enzyme utilization could save $3 to 5 billion dollar of feed cost per year.

The growth of feed enzyme market belongs to the adoption of phytase technology and the wider application of carbohydrases was done in corn-based diets. However, recently, international prices of major feed ingredients have been sharply increased by increasing demand for biofuel and severe drought in some areas such as U.S. and Europe. Therefore, many of new feed ingredients have been investigated and applied for livestock feed industry to replace the major feed ingredients. Although alternative ingredients may have benefit in relatively lower price, however most alternative materials contain high level of indigestible or anti-nutrients inherently.

Non-starch polysaccharides (NSP) are regarded as a major component of anti-nutritional factor in grains and by-products which is hardly indigested by monogastric animals. Each of feed ingredients has different NSP composition but major NSP can be sorted to α-galactoside, pentosans (arabinoxylan and xylan), β-mannan (Galactomannan) and β-glucan. Bach-Knudsen (2001) demonstrated that high fiber content of diets have contributed to block the access of digestive enzymes to the cell contents. O'Doherty and McKeon (2000) also reported that physical characteristics of NSP resulted increased passage rate of digesta and

reduced time to digest nutrient, causing reduced nutrient digestibility. Consequently dietary enzymes to degrade NSP fractions have been used frequently to improve the nutrient value of high NSP feed ingredients.

Generally, NSP enzymes showed more effective result in poultry diet, because digesta of poultry have higher viscosity than that of pig (Bedford et al. 1991). Effects of NSP enzymes on nutrient utilization and animal performance in poultry and weaner/grower pigs have been well documented by numerous researchers (Li et al., 1996; Yin et al., 2000; Diebold et al., 2004; Nortey et al., 2007; Olukosi et al., 2007a; Emiola et al., 2009; He et al., 2010; Reilly et al., 2010). Though Souza et al. (2007) reported improvement of dry matter and nitrogen utilization in ileal and total tract during lactation period with supplementary NSP enzyme, but information about effects of dietary NSP enzymes on sow performance was limited. Because fiber sources have been used frequently in gestating sow diet to increase of satiety and decrease of stress and stereotypic behavior of sows (Adeola and Cowieson, 2011). However, utilization of energy and nutrients is associated with development of fetus and sow performance, therefore dietary enzyme can contribute to improvement of their utilization.

During lactation period, sows may have negative energy and nutrient balance because lactating feed intake is insufficient to their nutrients requirement for milk production (Mullan and Williams, 1989; Yang et al., 1989). Therefore, supplementary NSP enzymes may have role in reduction of negative nutritional balance by improving additional utilization of nutrients. Moreover, effects of supplementary NSP enzyme can be much clearer in highly prolific breeding sow. Thus, supplementary NSP enzymes in gestating and lactating sow feed may provide economical feeding scheme according to an accurate count of additional nutrient availability.

Consequently, three experiments were conducted to evaluate the proper

application of NSP multi-enzyme (composed of galactosidase, galactomannanase, xylanase and β-glucanase) on sow performance including 1) estimation of energy-compensation effect of NSP multi-enzyme supplementation in gestating diet on physiological responses and reproductive performance, 2) investigation of the effects of NSP enzyme in lactating diet on physiological responses and reproductive performance of sows, 3) evaluation of the effects of dietary energy levels and enzyme supplementation in gestation diet based on nutrient digestibility.

Chapter II. Literature Review

1. Opportunities and challenges of exogenous enzymes to improve animal production

Exogenous enzymes have an important role in current feed industry (Choct, 2006). Today, almost 70% of wheat-barley based poultry feeds are provided with xylanases and β-glucanases, and over 90% of broiler diet in some countries such as UK, Canada and Australia includes feed enzymes. The potential benefits of exogenous enzymes are presented below.

1. Improve efficiency of dietary component utilization in feed ingredient
2. Increase flexibility in feed formulation to reduce feed cost
3. Reduce variability of ingredient quality
4. Improve uniformity of animals by uplifting growth performance
5. Improve intestinal morphology and microflora to enhance digestion and absorption of nutrient
6. Reduce excreta moisture content, and lower incidence of wet litter
7. Decrease manure output

Exogenous enzymes are expected to play key roles in feed industry because of increasing demand for meat production and economic efficiency. Feed enzyme technology has been rapidly developed with numerous researches. Currently, 3-phytase, 6-phytase, subtilisin, α-galactosidase, glucanase, xylanase, α-amylase and polygalacturonase are commercially available in pig and poultry feed formulation (Selle and Ravindran, 2007), and potential benefits of feed enzymes on aqua-culture and ruminant nutrition were evaluated (Beauchemin et al., 2003; Ringo et al., 2010).

During past years, feed enzyme technology was focused on production of enzymes rather than stability in digestive system with resistance to proteolysis during transit in gastrointestinal tract (Table 1). Today, Feed enzyme technology is investigating some new approaches such as improvement in aggressiveness toward their substrates for enzyme efficiency or enhancement of thermo-resistance for adding exogenous enzymes during feed processing like pelleting, as well as development of reliable and economical assays for measuring enzyme activity.

Table 1. Commercial enzymes from genetically modified microorganisms for cereal grains

Enzyme Activity	Host Organism	Doner Organism	Price ($/kg)
α-Amylase (Thermal)	Bacillus amyloliquefaciens Bacillus licheniformis	Bacillus sp. Bacillus sp.	1500-10.000
β-Glucanase	Bacillus amyloliquefaciens Bacillus subtilis Trichoderma reesei Trichoderma longibrachiatum	Bacillus sp. Trichoderma sp.	N/A
Glucose isomerase	Streptomyces lividans Streptomyces rubiginosus	Actinoplanes sp. Streptomyces sp.	N/A
Lipase	Aspergillus oryzae	Candida sp. Rhizomucor sp. Thermomyces sp.	202-206
Maltogenic amylase	Bacillus amyloliquefaciens Bacillus subtilis	Bacillus sp.	50-1500
Protease (Neutral)	Aspergillus oryzae Bacillus amyloliquefaciens Bacillus subtilis. Bacillus licheniformis	Rhizomucor sp. Bacillus sp. Bacillus sp.	3-30
Pullulanase	Bacillus licheniformis Klebsiella planticola	Bacillus sp. Klebsiella sp.	15-30
Xylanase	Aspergillus oryzae Aspergillus niger var. awamori Bacillus amyloliquefaciens Bacillus subtilis Bacillus licheniformis Trichoderma reesei Trichoderma longibrachiatum	Aspergillus sp. Thermomyces sp. Bacillus sp. Bacillus sp. Trichoderma sp.	10-80

2. Feed enzymes in livestock industry

2.1 Nomenclature and classification of enzyme

Basically, enzyme can be divided to endogenous enzymes and exogenous enzymes. Enzymes naturally are secreted in animal body refer as endogenous enzymes, and feed enzymes are classified to exogenous enzymes because they are produced from outside of the animals or segregated organisms in the body. The prefixes “endo-” and “exo-” also are used in the name enzyme which indicates the degrading site of target substrate by enzyme. Exo-enzymes degrade substrate from the terminal structural building blocks of molecular strand while endo-enzymes degrade structural bonds within the molecular strand.

Nomenclature of enzymes consists of 3 names of enzymes trival name, systematic name and EC number. Early discovered enzymes have arbitrary short names such as pepsin, trypsin, thrombin or lysozyme. Such names are called trival name, and these nomenclatures did not give information about substrate or function of enzyme. Systematic name of enzyme consist of names of substrates and type of reaction catalyzed by enzyme like lactate dehydrogenase. Enzyme often refers with recommended name which means the most common used name of enzyme.

Because of rapid discovery of enzymes, International Union of Biochemistry and Molecular Biology (IUBMB) proposed enzyme commission nomenclature in 1961. Today, the latest update had occurred in 1992 and a total of 3,196 enzymes were categorized by this rule. Enzyme commission number (EC number) is a categorization of enzymes by their catalyst reaction (Webb EC., 1992). Therefore, EC number does not specify enzymes, so different enzymes having similar catalyzing reaction could be categorized with same EC number. EC numbers consist of 4 parts of numbers with the letter EC. Top level of EC numbers consist of 6 classes including oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases from EC1 to EC6. The most of feed enzymes are belong to

EC3 group because digestive enzymes such as lipases, amylases, or peptidases catalyze hydrolytic reactions. For example, the enzyme catalyzing lactose to galactose and glucose has the trivial name lactase, the systematic name beta-D-galactoside galactohydrolase, and the classification number EC 3.2.1.23.

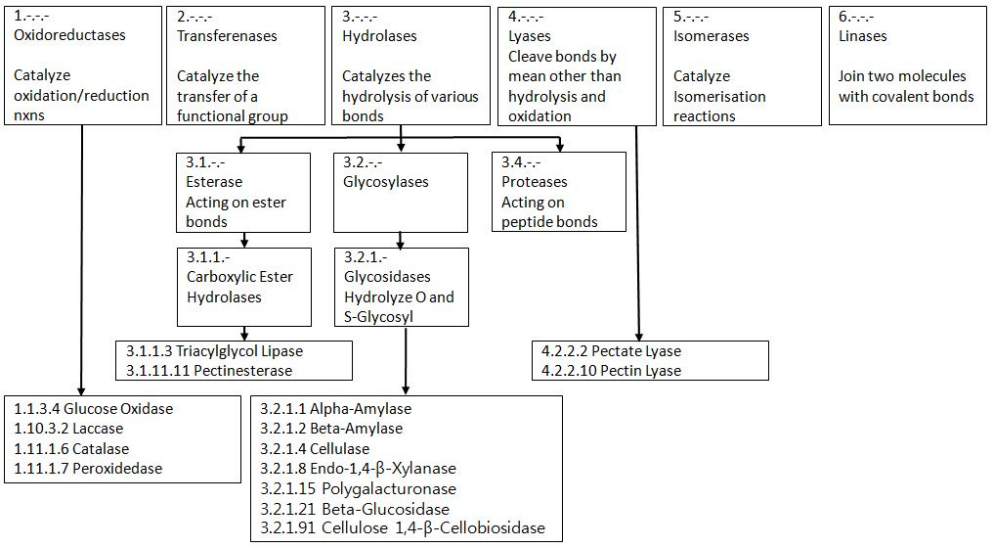


Figure 1. EC classification of enzymes (Sarrouh et al., 2012)

2.2 Enzymes used in animal nutrition

2.2.1 Carbohydrases

Plant-derived feed ingredients contain starch and non-starch carbohydrates. Carbohydrases break down these carbohydrates into simple sugars. Starch degrading enzymes (α-amylases) degrade starch in grains, grain by-products and some vegetable proteins. Inclusion of amylase potentially improves starch digestibility and extract more energy from feed ingredients. Additional amylase has benefit for young animals which has limited feed intake and pre-mature digestive system. Inborr et al. (1993) showed increased starch digestibility of early weaned pigs by addition of fiber and starch degrading enzyme. However, benefits of

additional starch degrading enzymes in feed diminished with ageing because starch is easily digested by mature monogastric animals.

Fiber is the most common anti-nutritional factor for monogastric animals. Fiber traps some of starch and proteins in cell wall (insoluble fiber) or dissolves in animal's gut and increase viscosity of digesta (soluble fiber). Moreover, fiber absorbs water in gut and interfere digestion of water soluble nutrient. High fiber contents in feed could make bulk in digestive track and reduce feed intake by increasing transition time (Dikeman and Fahey, 2006). The most common fiber degrading enzymes are xylanase and glucanase, and they account for 80% of carbohydrase market (Adeola and Cowieson, 2011). Endo-(1→3(4))-β-glucanases are often referred to as cellulases or glycosidases, but technically cellulase means a series of glycosidase degrade cellulose into glucose. Xylanases mainly break down arabinoxylans in plant, and often referred to as pentosanases, hemicellulases or NSP degrading enzymes. In addition, β-mannanase, α-galactosidase, and pectinase are commercially available in feed industry. Further details of non-starch degrading enzymes are described in Chapter II-3.

2.2.2 Phytases

Phytic acid is the major storage form of phosphate and inositol in almost all seeds (Reddy et al., 1982). Monogastric animals do not secrete sufficient endogenous phytase to breakdown the phytate molecule. Phytate holds approximately 70% of phosphorus in plant and poorly digestible to mono gastric animals (Eeckhout and DePaepe, 1994). Moreover, phytate forms complexes with calcium, proteins or starch which are undigested in gastro-intestinal track. Therefore, inclusion of phytase in feed increase availability of phosphorus in feed ingredients derived from plants as well as phytate-bond nutrients. Undigested phosphorus in feed excreted as manure, which can lead to environmental pollution

problems (Traylor et al., 2001). Today, phytase accounts for 60% of feed enzyme market. International Union of Pure and Applied Chemistry and the International Union of Biochemistry recognize two classes of phytases (3-phytase and 6-phytase). 3-phytase (Microbial phytase, EC 3.1.3.8) initially removes orthophosphate from the 3-position of phytic acid. 6-phytase (Plant phytase, EC 3.1.8.24) catalyzes the removal of orthophosphate from the 6-position of phytic acid. Beneficial effects of dietary phytase are very clear in many researches. Improvement of phosphorus bioavailability in various feed ingredients such as barley, corn, oat and wheat has been reported by several researchers (Bruce and Sundstol, 1995; Campbell and Bedford, 1992; Cromwell et al., 1995; Dungelhoef et al., 1974), and compensating effect of phytase with low phosphorus has also been introduced in swine and poultry studies (Simons et al., 1990; Yi et al., 1996). Recently, several researches represented growth promoting effect of supplemental phytase in pigs (Kies et al., 2006; Brana et al., 2006) and broiler (Shirley and Edwards Jr., 2003; Walk et al., 2012) studies when overdose of phytase was performed.

2.2.3 Proteases

Proteases degrade storage proteins in plant-derived ingredients. Storage proteins originated from seed production and provide amino acids for germination. Especially, legume seeds contain much amount of storage proteins binding starch. Therefore, supplemental proteases break down these storage proteins in feed ingredients and increase available starch contents. Also, protease reduces protein inhibitors such as trypsin inhibitor or lectins. Tamminga et al. (1995) presented that presence of soybean trypsin inhibitor caused inactivation and hypersecretion of pancreatic proteolytic enzymes, which induced subsequent loss of endogenous protein in monogastric animals.

Proteases are commercially added in swine and poultry diet with the other

enzyme complex, and several studies were conducted. Jo et al. (2012) represented growth promoting effect of protease on growing pigs when protease added with multiple carbohydrase in corn-soybean meal based diet, and Souza et al. (2007) demonstrated improvement of dry matter digestibility and nitrogen retention of sows when protease and xylanase were added in sow diet. However, effects of inclusion of protease enzymes in animal diet are inconclusive (Ghazi et al., 1997). In the study of Ghazi et al. (1997), they compared two types of proteases isolated from Bacillus species or Aspergillus species, only later protease improved nutritional value of soybean meal. In addition, Caine et al. (1997) failed to show positive effects of protease treated soybean meal on weaning pig, and Rooke et al. (1998) represented that *in vitro* assessment did not predict responses of protease *in vivo*.

Table 2. Commercial enzymes for target substrate and feedstuffs

Enzyme	Target substrate	Target feedstuffs
β-Glucanases	β-Glucan	Barley, oats and rye
Xylanases	Arabinoxylans	Wheat, rye, triticale, barley, fibrous plant materials
α-Galactosidases	Oligosaccharides	Soybean meal, grain legumes
Phytases	Phytic acid	All plant-derived ingredients
Proteases	Proteins	All plant protein sources
Amylases	Starch	Cereal grains, grain legumes
Lipases	Lipids	Lipids in feed ingredients
Mannanase Cellulases Hemicellulases Pectinases	Cell wall matrix (fiber components)	Plant-derived ingredients, fibrous plant materials

2.3 Factors affecting enzyme efficiency

Although both of pigs and poultry are categorized as monogastric animals, they have very different digestive tracks. Retention time of feed in the gastrointestinal track is 2~4 hours for chicks, and 12~24 hours for pigs (Entringer et al. 1975; Mateos et al. 1982; Johansen et al. 1993). Similarly, gastric phase of consumed feed may last 20~45 minutes and 4~8 hours for chicks and pigs, respectively. This difference of retention times in gastrointestinal track notably affects response of supplemented enzymes (Bedford and Schulze, 1998). Because of relatively short digestive track, digesta of poultry is much watery than pigs. Therefore, viscosity of digesta is much higher in poultry than pigs when fed similar diets (Bedford et al. 1991).

Structure of substrate is one of the most important factors to design NSP enzymes. Basic structures of xylans are similar, but backbone size and type and degree of substitutions are very different from sources of xylans such as hardwood, softwood or cereal xylans. As likely as xylans, backbone and substitution of β-glucans can be varied which affect physical properties such as solubility, water binding capacity and viscosity as well as susceptibility to enzymes (Ogawa, 1988; Bastawde, 1992). Therefore, design of enzyme needs considerations about their sources. For example, effective xylanase requires (1→4)-β-endoxylanase for cleaving internal linkage of backbone, β-D-xyloxidase which hydrolyzes short oligosaccharides to release of xylose. And other enzymes were designed to release other substituents.

Exogenous NSP enzymes are generally produced from fungi or bacteria. Sunna and Antranikian (1997) compared the characteristics of various endoxylanases produced from different 31 fungi with 31 bacteria (Table 3). Theses variations represent different environments for effective enzyme activity.

Table 3. Variation of characteristics of endoxylanases form from 31 species of bacteria and fungi (Sunna and Antranikian, 1997)

Organism	Optimal temperature	Optimal pH	Molecular weight	Vmax (U/mg)	Km (mg/ml)
Fungi					
Min	30℃	2.0	13,000	3.7×10^{-1}	0.09
Max	80℃	7.0	95,000	1.4×10^{4}	22.3
Bacteria					
Min	30℃	4.5	13,200	1.7×10^{-2}	0.07
Max	105℃	10.0	350,000	7.6×10^{3}	17.7

3. Non starch polysaccharides (NSP) in feed ingredients

3.1 Characteristics of non starch polysaccharides

The term of non starch polysaccharides (NSP) covers a large variety of polysaccharide molecules excluding starch (Choct, 1997), but in animal nutrition, NSP can be referred to polysaccharides which cannot be digested by monogastric animals.

Non starch polysaccharides are found mainly in cereal grains such as wheat, rye, triticale, barley and cereal byproducts including wheat bran, rice bran and palm kernel meal (Figure 2). Addition of NSP degrading enzymes in the feed can help releasing more available nutrients to absorb in the small intestine because pigs cannot secret endogenous NSP degrading enzymes.

The most important nature of NSP is water solubility because actions of soluble and insoluble NSP in digestive track of monogastric animals are very different. Because of the ability of soluble NSP to increase viscosity of digesta, negative correlation between NSP content in feed ingredients and nutrient utilization has been clearly demonstrated in poultry (Choct and Annison, 1990; Annison, 1991) and pigs (King and Taverner, 1975).

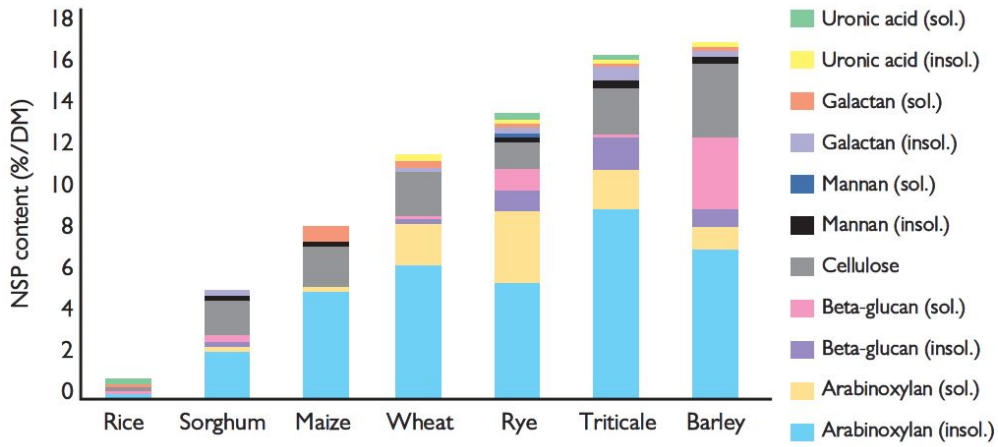


Figure 2. NSP in selected feed stuffs (International Pig Topics Vol. 22, 2007)

Increase of digestive track viscosity decreases diffusion rate of substrates and interaction with digestive enzymes at the mucosal surface (Edwards et al., 1988; Ikegami et al., 1990). Soluble NSP interacts with the glycocalyx of intestinal brush border and reduces nutrient absorption (Johnson and Gee 1981). The anti-nutritive effect of NSP has been supported by the widely use of dietary NSP enzymes in monogastric animal feeds. NSP enzymes cleave large NSP molecules into smaller polymers, which improve nutrient utilization and reduce gut viscosity in animal digestive system (Bedford et al., 1991; Choct and Annison, 1992).

In contrast, insoluble NSPs have ability to absorb large amounts of water and maintain the consistency of the excreta in monogastric animals (Stephen and Cummings, 1979). Although there are some argues that insoluble may result in low nutrient digestibility because of shorten residence time of digestas in gut, insoluble NSP has little effect on nutrient utilization in monogastric animals (Begin, 1961). Furthermore, there are lots of evidences to suggest that feed ingredients containing insoluble fiber enhances gut development of monogastric animals (Choct, 2006; Hetland et al., 2007).

3.2 Target of NSP enzymes in feedstuffs

3.2.1 Major NSPs in feed ingredients

Cellulose

Cellulose is a linear homopolymer of (1→4)-β-glucose units. In plant ingredients, linkage up to 7,000-10,000 glucose units can be found (Goring and Timell, 1962). Individual cellulose chains lie side by side in bundles and form twofold helix combined by hydrogen bonds between neighboring -OH groups (Gardner and Blackwell, 1974).

Cellulose is the most abundant biopolymer in nature, and believed that cellulose is identical in chemical composition regardless of sources. Cellulose is insoluble in water or alkali solutions (Table 4).

Table 4. Enzymes involved in lignocellulose degradation (Banerjee et al., 2010)

Fraction	Enzymes	Location of action	E.C number
Cellulose	Endo-1,4-β-glucanases	Cellulose (amorphous regions)	3.2.1.4
	Cellobiohydrolases	Cellulose (crystalline regions)	3.2.1.91
	β-glucosidases	Cellobiose, cellodextrins	3.2.1.21
Hemicellulose	Endo-xylanase	Xylan main chain	3.2.1.8
	Exo-xylanase	Xylan main chain	3.2.1.37
	β-Xylosidase	Xylooligosaccharides	3.2.1.32
Lignin	Laccase (phenol oxidase)	Phenolic compounds in lignin structure	1.10.3.2

Pentosans (xylans, arabinoxylans)

The basic units of cereal pentosans (arabinoxylans) are arabinose and

xylose. Molecular structure of pentosan consists of a linear (1→4)-β-xylan backbone with arabinose residue which attaches through O_2 and O_3 atoms of xylosyl residues (Perlin, 1951). Though major substituents of the backbone are single arabinose residues, but hexoses and hexuronic acids are observed as minor constituents (Fincher, 1975). In this basic structure, phenolics and proteins can be attached as side chains (Geissmann and Neukom, 1973; Neukom, 1976).

Wheat and rye contain high level of arabinoxylans. Arabinoxylans are predominant cell wall storage polysaccharides in cereal grains and located in the cell wall of stachy endosperm and aleurone layer. Pentosans are anchored in the cell walls by alkali-labile ester-like cross links, so the most of the arabinoxylans in cereal grains are insoluble in water (Mares and Stone, 1973). However, unbound arabinoxylans to the cell walls can be absorbed 10 times of water of their weights and forms highly viscous solutions. Arabinoxylans rapidly develop gel network when peroxide oxidative agents are present which led digestive disorder (Geissmann and Neukom, 1973) Pentosans are major component reducing metabolic energy of wheat in animal (Choct and Annison, 1990), and also decreases utilization of nutrients such as starch, proteins and fat.

β-glucans

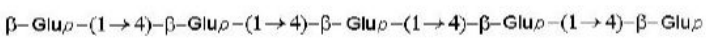
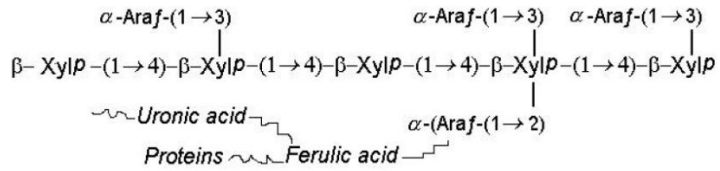
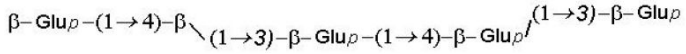
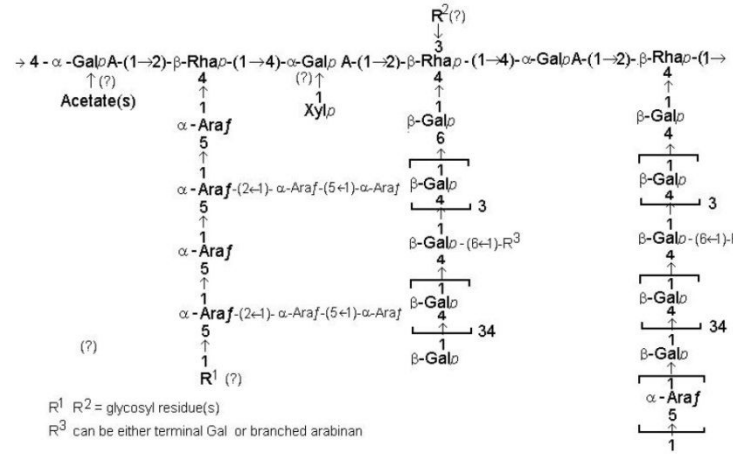
The cereal ß-glucans are soluble mixed-linkage (1→3), (1→4)-β-D-glucans. β-glucans are predominant cell wall polysaccharides with arabinoxylans in cereal grains located in endospermic cell walls and aleurone layer. β-glucans can be found in most of cereal grains, being particularly high in barley and oats. Although the β-glucans and cellulose are comprised of β-linked glucose units there is little similarity in their physical properties. In barley, the β-glucans consist of 70% 1,4 linkages and 30% β-(1→3) linkages. Generally, two or three segments β-(1→4) are separated by single β-(1→3) linkages (Parrish et al. 1960). However, up to five

contiguous β-(1→3) linkages exist as minor structural features (Fleming et al. 1974). The β-(1→3) linkages are broken up uniformity of β-D-glucan molecule structure which brings flexibility and water solubility. β-glucans have been known to anti-nutritional factor (Burnett, 1966), but recently, β-glucans focused as immune stimulators in young pigs to enhance susceptibility towards infections through increasing glucocorticoid secretion (Hiss and Sauerwein, 2003).

Mannan

Mannans are consist of a (1→4)-β-mannan backbone substituted with galactose or glucose (Carpita and McCann, 2000). Galactomannans present as the major non-cellulosic hexosans in copra and palm kernel meal, and endospermic legumes, such as guar and locust bean, contain galactomannans in the endosperm during seed development (Meier and Reid, 1977). Mannan is considered as anti-nutrient due to its viscous property. Unavailable mannan in feed ingredients increases viscosity of feed and reduces the post prandial glucose and insulin levels (Jenkins et al., 1977). Mannan also decrease amino acid absorption in small intestine of rat (Elsenhans et al., 1980), and interfere glucose absorption in small intestine of growing pig (Sambrook and Rainbird, 1985). Therefore, high level of mannan in feed reduces growth performance of animal.

Table 5. Typical structures of NSP in feed (Choct, 1997)

NSP	Structure
Cellulose	
Arabinoxylan	
β-glucan	
Pectic polymer	

3.2.2 Target of NSP enzymes

Role of NSP enzyme is not to degrade NSPs into simple sugars, but a cleaving of NSPs into smaller polymers to prevent formation of viscous digesta. Use of dietary NSP enzymes needs consideration of viscosity-inducing molecules in feed ingredients. Benefits of enzymes follow the quantity of NSP in feed ingredients which mean same enzyme have different efficacy to different part of plant such as wheat and wheat bran. Wheat bran which has plenty NSP, also shows the highest improvement in nutrient digestibility with inclusion of xylanase.

Some feed grains like corn and sorghum contain little amounts of soluble NSP. These materials do not produce viscous digesta when fed to monogastric animals. Therefore, benefits of dietary enzymes for reducing viscosity and increasing available nutrient can be used in diets containing these grains (Figure 3). As mentioned before, 80% of the global carbohydrase market is accounted for 2 dominant enzymes, xylanase and glucanase.

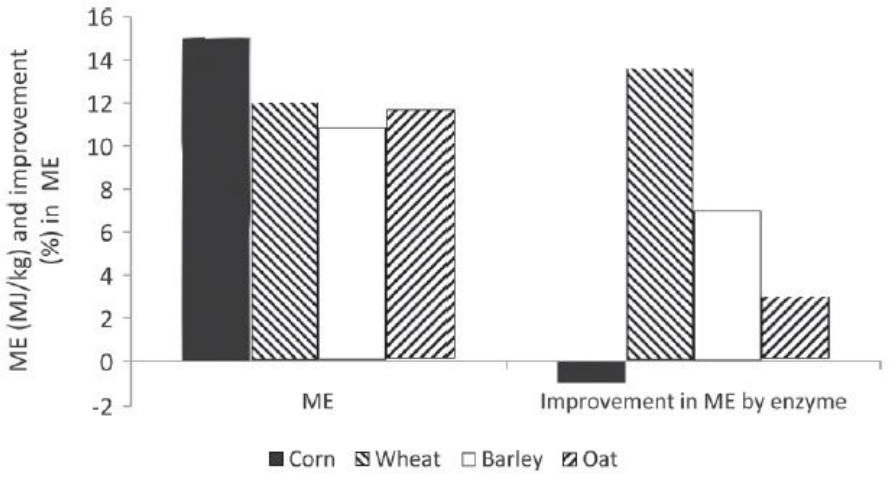


Figure 3. Relationship between supplementary xylanase and improvement of energy in selective feed ingredients (Palander et al., 2005)

In the diet based on wheat, rye or triticale, the major NSP inducing anti-nutritive activities in monogastric animal is arabinoxylan. Wheat by-product such as wheat middling contains even greater amount of NSPs than wheat grain. The arabinoxylans are not bound to the cell walls and can form highly viscous solutions and absorb water in gut. Benefits of additional xylanase in poultry and pig diet are well established. Addition of xylanase reduces gut viscosity (Table 6), and increases nutrient digestibility (Bartelt et al., 2002), growth performance and feed efficiency (Bedford et al., 1991; Bedford and Classen, 1992; Pettersson and Aman, 1988). Studies about xylanase with a triticale based diet on pig performance are rare, but

improvement in metabolic energy of broiler diets were reported. Triticale contains similar amounts of arabinoxylans and β-glucans. Therefore, combination of xylanase and β-glucanase can be more effective in the triticale based diet.

Table 6. Viscosity[1] (mPa/s) of digesta of growing pigs fed rye-wheat diet with or without xylanase (Bartelt et al., 2002)

	Rye-wheat	Rye-wheat + Xylanase	P-value
Duodenum	1.52 ± 0.05	1.41 ± 0.04	ns
Ileum			
0-3h	5.09 ± 1.11	2.06 ± 0.36	< 0.05
3-6h	11.79 ± 2.67	3.18 ± 0.44	< 0.05
6-9h	13.38 ± 2.66	4.96 ± 1.33	< 0.05
9-12h	12.91 ± 7.34	2.82 ± 0.56	ns

[1] Share rate: duodenal digesta = 450 s^{-1}; ileal digesta = 45–450 s^{-1}

Barley or oats contains high level of β-glucan (3-4%). β-glucan levels related to reduced nutritional value of feed containing barley or oat (Burnett, 1966). Therefore, addition of β-glucanases especially in a barley based diet successively improves performance of monogastric animal (Campbell et al., 1989). However, barley also contains notable amount of soluble NSP. Therefore, addition of combination of β-glucanase and xylanase are common in barley based diet.

Inclusion of cereal by-products such as wheat bran or rice bran is limited below 10% because of high level of NSPs in monogastric animals. Therefore, various enzymes including xylanase, ß-glucanase and cellulose have been introduced to improve nutrient utilization of cereal by-products for pigs and poultry, and showed some improvement of nutritive value of diets containing high level of cereal by-product (Choct, 2006). However, conclusive evidence that NSP enzyme could degrade insoluble NSPs such as cellulose or mannans within gut transition

time had not been proposed by several researches. DDGS(Dried Distiller's Grains with Solubles) is also a major ingredient in feedstuff. Corn DDGS contains up to 40% carbohydrates which are divided into similar amount of NSP and starch (Choct and Petersen, 2009), but nutrient and energy utilization of DDGS are varied (Péron and Partridge, 2010). DDGS contains high level of insoluble NSPs, so effective enzymes for DDGS need degrading ability of insoluble NSP, such as cellulose and arabinoxylans. Also, enhanced nutrient digestibility and improved growth performance in poultry and swine (Emiola et al., 2009) were reported when an enzyme cocktail (xylanase, amylase, protease and phytase) were added in a DDGS rich diet.

Copra or palm kernel meal is cheaper ingredient than conventional feedstuffs but inclusion in monogastric animal diets is very limited due to high level of mannans (Purwadaria et al., 1995). However, this limitation has been solved by development of commercial ß-mannanase. Copra meal consists of 45-60% NSP and palm kernel meal also contains at least 60% of NSP. In case of palm kernel meal, 78% of total NSP consisted with insoluble linear mannans. Sundu and Dingle (2003) demonstrated that 3 enzymes including ß-mannanase, galactosidase and celullase are needed to improve nutritive value of palm kernel meal.

4. Benefits of NSP enzymes for animal nutrition

4.1 NSP enzymes on gut status

The role of NSP enzymes is to hydrolyze complex indigestible carbohydrates in feed ingredients. Degradation of NSPs in feed materials is directly related to the decrease of viscosity of digesta, and reduction of digesta viscosity can be the most important role of NSP enzyme supplementation (Vahjen et al., 2007).

In the study of Nitrayova et al. (2009), addition of 200 mg xylanase per kg of rye based diet decreased presence of ileal NSP and xylose contents about 114%

and 740% respectively. Xylanase degrades arabinoxylan backbone of pentosans and depolymerizes to oligomer (Bengtsson et al., 1992; Courtin and Delcour, 2002; Hu et al., 2008). Although, liberation of oligomer still did not mean digestible, Adeola and Bedford (2004) showed that inclusion of xylanase had better response in high viscosity wheat than low viscosity wheat. This observation indicates that one of benefits of supplementary xylanase is to reduce NSP induced digesta viscosity (Figure 4).

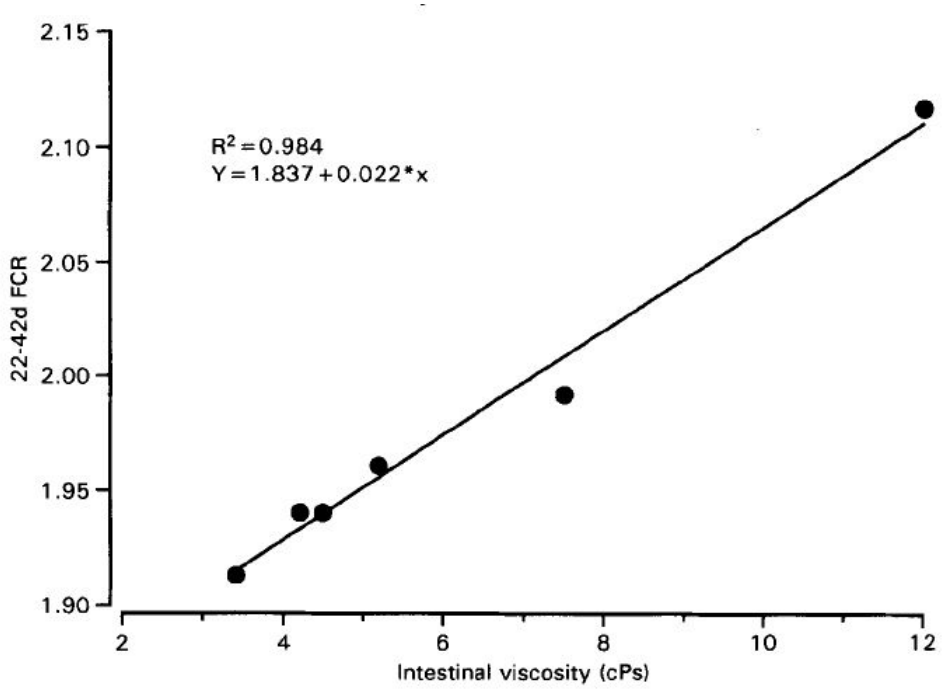


Figure 4. Relationship between FCR and Intestinal viscosity of broiler with addition of xylanase at from 0 to 3000 U/kg in wheat based diet (Bedford and Schulze, 1998).

Viscosity of digesta delays diffusion rate and shedding of microorganism which induce proliferation of harmful microorganisms (Meyer et al., 1986; Vahjen et al., 1998). McDonald et al. (2001) demonstrated that supplementation of diet

inducing viscous digesta increased shedding of enterotoxigenic *E. coli* in weaning pigs. Moreover, Teirlynck et al. (2009) reported that broilers fed wheat-rye had higher incidence of apoptosis, immunological status and microbial invasion in small intestine than broilers fed corn-based diets. Generally, digesta viscosity affects more critically in poultry than in swine.

High level of NSP in diet induces poor gut health and morphology of the digestive surfaces also can be accrued. Mathlouthi et al. (2002) showed that supplementation of xylanase in rye based diets improved gut morphology of broiler, and McDonald et al. (2001) reported decrease of small intestinal villus height along with decreased empty body weight gain when weaning pigs provided higher NSP diet.

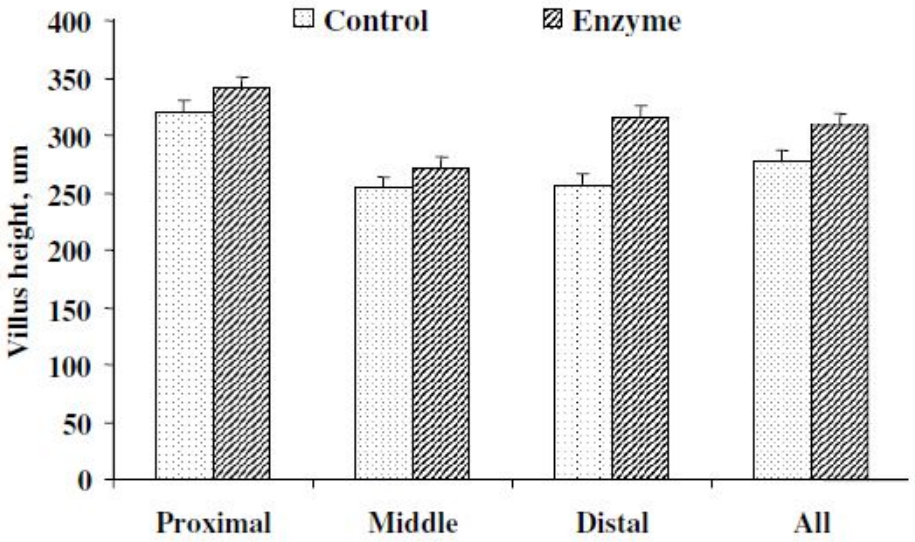


Figure 5. Effect of enzyme mixture on villus height of young pigs (Kim et al., 2003)

Supplementation of NSP enzyme modifies gut microflora. *Lactobacillus* inhibits the growth of harmful bacteria such as pathogenic E. coli in the gut of animal. Vahjen et al. (1998) reported increase of intestinal *lactobacilli* and

reduction of digesta viscosity when xylanase was added in wheat based broiler diet. Similarly, Hampson et al. (2002) showed improved intestinal microbial population in layers when layers fed xylanase and protease.

Kiarie et al. (2007) observed increase of lactic and organic acids production and decreasing ammonia production when early weaned pigs fed additional NSP enzymes. Increase of lactic acid production in gut suppresses the growth of pathogens (Pluske et al., 2001). Furthermore, xylose produced during hydrolysis of arabinoxylans is known to improve growth of beneficial bifidobacteria (He et al., 2010).

Consequently, addition of NSP enzyme induces hydrolysis of NSP and helps domination of beneficial bacteria in digestive track of target animal.

4.2 NSP enzymes and nutrient utilization

Effects of NSP enzymes on nutrient digestibility are not consistent. Several researchers presented benefits of supplementary NSP enzymes on the digestibility of dry matter (Li et al., 1996; Nortey et al., 2007; Olukosi et al., 2007a), energy digestibility (Yin et al., 2000; Diebold et al., 2004; Olukosi et al., 2007a), nitrogen (Yin et al., 2000; Emiola et al., 2009; Reilly et al., 2010) or minerals digestibility(He et al., 2010). However, other researchers did not observe improvement in dry matter (Woyengo et al., 2008), crude protein (Nitrayova et al., 2009) or mineral (Olukosi et al., 2007b; Nortey et al., 2008) digestibility.

Increase of energy utilization with supplementary enzyme is reasonable because NSP enzymes help release of energy-yielding nutrient captured in NSPs. NSP contents in feed materials reduce energy utilization of animal for various reasons. High level of NSPs in diet reduces feed intake because of their bulkiness. In addition, NSPs interrupt substrate-enzyme contact, and increase endogenous energy loss and decrease nutrient absorption.

Although some of undigested NPS fraction can be utilized by fermentation of microorganisms in large intestine, but shifting of digestion from distal to proximal intestine greatly increases absorption efficiency of nutrients. Several researchers have reported increase of monosaccharide or oligosaccharides in ileum with supplementation of NSP enzymes such as xylanase (van der Meulen et al., 2001), β-glucanase (Li et al., 1996) and multi-carbohydrase (Kiarie et al., 2007). Increase of broken products in ileum indicates degradation of NSP, and improves energy utilization through absorption of monosaccharides in proximal intestine.

Furthermore, consumption of high NSP induces cell proliferation and turnover in gastrointestinal track. Johnson and Gee (1986) observed decrease of DNA and protein contents of the intestinal brush border by feeding rats with diet contained high level of NSP. Thus, degradation of NSPs with NSP enzymes could reduce energy partitioning which dissipated in cell turnover.

Several researchers observed improvement of fat and starch digestibility by addition of dietary xylanase or β-glucanase supplementation (Adeola and Bedford, 2004; Tervila-Wilo et al., 1996; Vahjen et al., 2007). Improvement of fat digestibility is reasonable because NSP contents hydrolyze bile salts and reduce fat utilization in animal (Mathlouthi et al., 2002).

In addition, digesta viscosity affects energy utilization. Macleod et al. (2008) reported 5% increase of ME in naked oat, and Adeola and Bedford (2004) reported 4% increase of ME when digesta viscosity was decreased 250% and 70% respectively. These results indicate that decrease of digesta viscosity by supplementary NSP enzyme enhance energy utilization.

Fiber contents in feed ingredient related to nitrogen digestibility for low availability of nitrogen as well as excretion of nitrogen captured in the fiber (Stanogias and Pearcet, 1985). NSP enzymes degrade indigestible NSP in feed ingredients and increase chances for accession of trapped protein and digestive

proteases.

Several studies reported improvement of amino acid digestibility with NSP enzyme supplementation in wheat based diet (Diebold et al., 2004; Barrera et al., 2004; Vahjen et al., 2007) and barley-based diets (Li et al., 1996). One reason of improved nitrogen or amino acid digestibility induced by reduction of endogenous amino acid losses. NDF and ADF have been known to increase endogenous and microbial nitrogen loss. Dietary NSP induces damage on mucosa and loss of pancreatic enzymes and bile acid which cause endogenous nitrogen and amino acids losses (Schulze et al., 1995). Nitrogen loss by fibrous diets can be increased about 59% and 41% for endogenous and exogenous nitrogen respectively.

Although, supplementation of NSP enzymes have role in improvement of nitrogen and amino acids utilization in feed ingredients, but results of studies reporting endogenous protein loss or amino acids digestibility are not consistent (Figure 6).

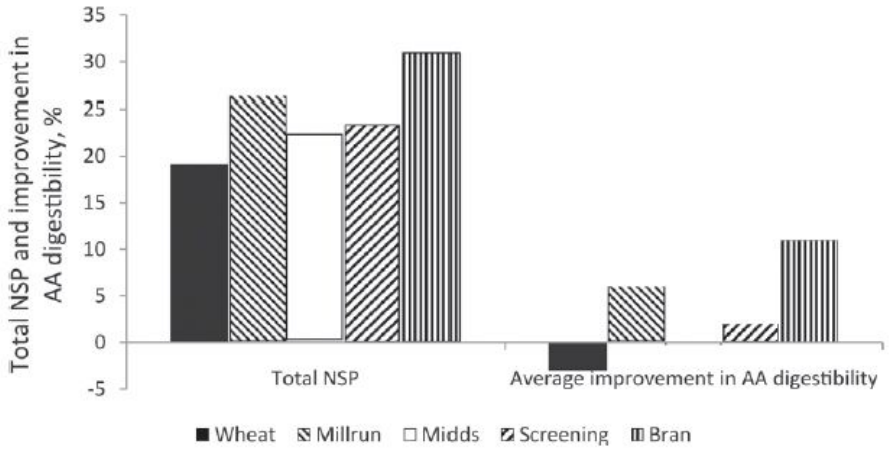


Figure 6. Effect of additional xylanase in wheat fractions on improvement of amino acid digestibility in pigs (Nortey et al., 2008)

Barrera et al. (2004) demonstrated that supplementation of xylanase

improved amino acid digestibility by 11%. However, Rutherfurd et al. (2007) did not observe improvement of true ileal amino acid digestibility with commercial enzymes including xylanase, α-amylase, and β-glucanase. Such results can be obtained by feed type, ingredient or presence of certain anti-nutritional factor. Underestimation of dietary NSP enzymes on nitrogen or amino acid digestibility may be resulted by presence of intact cell walls when enzymes do not target specific bonds of substrates.

Addition of NSP enzymes indirectly affects amino acid digestibility. As other nutrients, decrease of digesta viscosity improves nitrogen and amino acid absorption. In addition, increase of starch digestibility by supplementary enzymes indirectly affects nitrogen or amino acid digestibility. Poor absorption of glucose reduces amino acid transport because glucose has role in regulating amino acids transport pathways and protein synthesis (Roos et al., 2009).

As other nutrients, supplementation of NSP enzyme improves utilization of minerals. Nortey et al. (2008) observed reduced mineral digestibility with high NSP ingredients. Previous researchers demonstrated improvement of phosphorus digestibility with of xylanase (Nortey et al., 2007) or enzyme complex (Olukosi et al., 2007a). The most of phosphorus is bound in phytic acid, and found in aleurone layer (Frolich, 1990). Parkkonen et al. (1997) represented that NSP enzymes increase permeability of the aleurone layer which induced release of unavailable minerals. Consequently, effect of dietary NSP enzymes on phytic acid and other minerals can be resulted from exposing to endogenous enzymes during degradation of substrates.

4.3 NSP enzymes and swine performance

Improvement of nutrient digestibility by supplemented enzymes does not fully explain all the effects of NSP enzymes on growth performance of

animal(Table 7).

In the study of Barrera et al. (2004), pigs fed low amino acid diet with additional crystallized amino acid showed much higher improvement of growth than pigs fed low amino acid diet with NSP enzymes. Effects of dietary NSP enzymes on growth performance of pigs have not been consistent. Several researchers introduced growth promoting effect of supplementary enzymes in high NSP diets (Cadogan et al., 2003; Barrera et al., 2004; Kiarie et al., 2007), but the other researches failed to show the benefit of enzymes on body weight gains (Inborr et al., 1994; Mavromichalis et al., 2000; Olukosi et al., 2007b; Woyengo et al., 2008).

Effect of dietary enzymes is known to give more benefits in younger pigs because of their limited gut capacity and available nutrients (Adeola and Cowieson, 2011). Omogbenigun et al. (2004) demonstrated the consistent improvement in growth performance with NSP enzymes in wheat-soybean meal based diet, but later they did not observe any improvement in growth performance with same enzymes (Omogbenigun et al., 2007).

In case of a sow study, Souza et al. (2007) reported that supplementation of NSP enzymes improved ileal and total tract dry matter and nitrogen utilization during lactation period. Effects of dietary NSP enzymes on sow performance are varied. Adeola and Cowieson (2011) mentioned that little information about enzymes with sow provably due to the importance of fiber in sow diet.

Table 7. Summary of effects of exogenous NSP enzymes on growth performance of pigs (Adeola and Cowieson, 2011)

Reference	Animal	Feedstuffs	Enzyme	Observation
Barrera et al., 2004	Growing	Wheat	Xylanase	ADG 15%↑
Emiola et al., 2009	Finishing	Corn, barley, DDGS	Multiple	ADG 15%↑
He et al., 2010	Weaning	Corn, wheat, wheat bran	Xylanase	ADG 20%↑
Mavromichalis et al., 2000	Weaning	Wheat	Xylanase	No effects
Mavromichalis et al., 2000	Finishing	Wheat	Xylanase	Inconsistent
Olukosi et al., 2007	Nursery	Corn, wheat, rye	Xylanase	No effects
Olukosi et al., 2007	Growing	Wheat, wheat middling	Xylanase	No effects
Vahjen et al., 2007	Weaning	Wheat, wheat bran	Multiple	ADG 6%↑
Vahjen et al., 2007	Weaning	Wheat, wheat bran	Xylanase	ADG 7%↑
Woyengo et al., 2008	Growing	Wheat	Xylanase	No effects

4.4 Matrix value and feed formulation using NSP enzyme in swine

Matrix values like nutrient-equivalent values are assigned to enzyme product in least-cost formulation. These values demonstrate the amount of additional nutrients and economical advantage of enzymes during feed formulation.

Animal feed producers add NSP enzymes in feeds with two different purposes. The first purpose is to enhance nutrient utilization of standard feed with enzymes for expecting higher animal performance. And the other purpose is to use enzymes for saving feed cost. In the least-cost formulation, the greatest cost pressure is available or digestible energy content which is derived from dietary fat and starch (Hicks et al., 2001). Producers can reduce total amount of energy and nutrients in feeds by improving nutrient utilization with enzymes. During this procedure, conventional feed ingredients such as corn, soybean meal, wheat or barley change to relatively cheap by-product ingredients with addition of NSP enzyme (Bedford and Partridge, 2010). In this case, prediction of proper matrix values of NSP enzyme is essential to quantify the benefits from enzyme

supplementation, and amount of additional available nutrients by supplemental enzyme need to be calculated before enzymes are included in feed formulation. Inappropriate matrix value of enzymes could depress growth and performance of animals or increase excretion of nutrients. If its matrix value is too small, the enzyme effect is masked, whereas if the matrix value is too large, the diet remains nutritionally inadequate. Prediction of matrix values can be obtained from *in vitro* or *in vivo* trials. Matrix value of enzyme depends on feed ingredients, so can be diverse between feed formulations.

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Chapter III: Energy Modulation and NSP Enzyme Supplementation in Gestation Diet on Physiological Responses and Reproductive Performance of Sows

ABSTRACT: This experiment was conducted to investigate the effects of dietary energy levels and enzyme supplementation in gestation diet on physiological responses and reproductive performance of sows. A total of 48 multiparous sows (F1, Yorkshire x Landrace; Darby, Korea) with an initial BW of 216.9 ± 51.6 kg were allotted to one of four treatments with a 2 x 2 factorial arrangement. The first factor was energy levels in diet (3,165 or 3,265 kcal of ME/kg), and the second factor was inclusion of NSP enzyme complex (α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea). The experimental diets containing different energy levels and with or without supplementation of 0.1% NSP enzyme were supplied in gestation period. All other nutrients were met or exceeded the requirements of NRC (1998), and sows were fed 2.4 kg of diet daily during gestation. During lactation, the same diet was provided *ad libitum* regardless of dietary treatments with a free access to water. During the whole experimental period, there were no significant differences in the results of body weight, backfat thickness, and ADFI in lactation. Even though the significant effects were not detected on body weight, backfat thickness and ADFI during lactation, sows fed diets containing NSP enzyme showed numerically reduced body weight change(0-21 d). In the results of reproductive performance, litter growth and the average weight of piglets at birth were elevated when sows were fed high energy (energy response, $P<0.01$) and enzyme diets (enzyme response, $P<0.03$). When sows were fed diet containing 3,265 kcal of ME/kg with 0.1% NSP enzyme showed numerical decrease of litter size without significant difference. Increased plasma concentrations of progesterone at mating and insulin

at 70 d were observed when NSP enzyme was added to gestation diet (enzyme response, $P<0.05$), and decreased plasma concentration of glucose at 110 d was detected when dietary energy level increased (energy response, $P<0.05$). The colostrum and milk compositions such as milk fat, protein, total solid and solids-not-fat were not significantly different as dietary treatments were changed. The content of lactose at 7 d of lactation was increased when NSP enzyme was added to gestation diet (enzyme response, $P<0.05$). Consequently, feeding diet containing 0.10% NSP enzymes regardless of energy levels had beneficial effects on body weight and back fat thickness change of sows, and those effects of supplementing NSP enzyme was increased as dietary energy level decreased.

Key words: Feed, Energy, Enzyme, Gestation, Lactation, Sow

INTRODUCTION

Pigs like other monogastric animals cannot produce NSP enzymes, but micro-organism in the intestine can digest a small amount of NSP by fermentation. Monogastric animals have poor digestibility of NSP, subsequently anti-nutritional factors can affect the utilization of other nutrients. The NSP is mainly composed of mannan, β-glucan and xylan. Many findings demonstrated that the addition of NSP enzymes to the diet had influenced on improving digestibility of NSP (Li, 1996; Jackson, 1999). For increasing satiety, decreasing stress and stereotypic behavior of sows, the fiber sources have been used frequently in gestation diet, but high content of NSP causes digestive problems. However, the effect of NSP enzyme on the digestibility of gestating sows was smaller than that of growing-finishing pigs, because restriction feeding method has been applied usually for gestating sows.

Dietary energy intake in gestation is associated with the development of fetus and the body conditions of sows in lactation, moreover, sow body condition is a major factor for reproductive performance and longevity. Dourmad (1996) presented that proper energy intake of gestating sows was essential to decrease body weight loss in lactation, and recommended level of energy for gestating sows is 8,500 kcal of ME daily. Kongsted et al. (2004) demonstrated that low energy diet during gestation decreased reproductive performance of sows, and the negative effect of high energy diet on implantation of fetus, voluntary feed intake and obesity of sows also has been reported by several researchers (Dourmad et al, 1991; Weldon et al, 1994). However, little information is available about the supplementation effects of NSP enzyme on gestating sows. Recently, the findings to evaluate the effects of NSP enzyme has been concentrated on piglets because the digestive system of piglets is incomplete compared with those of adult pigs.

Therefore, this experiment was conducted to investigate the effects of dietary NSP enzyme on physiological responses and reproductive performance of sows.

MATERIALS AND METHODS

Animal Housing and Treatments

A total of 37 multiparous sows (F1, Yorkshire x Landrace, 4.9 ± 1.6 parity; Darby, Korea) with an initial BW of 216.9 ± 19.6.6 kg were used for a trial, at a research farm located in Eum-seong, Korea. The experiment was designed as a 2 x 2 factorial arrangement of treatments, and dietary energy levels and enzyme levels were main factors. Sows were allotted to each treatment based on body weight and backfat thickness by completely randomized design (CRD) after mating and were housed in an individual gestation stall (2.20 x 0.65 m^2) until 110 d of gestation. On 110 d of gestation, sows were moved into environmentally controlled farrowing rooms and placed in individual farrowing crate (2.50 x 1.80 m^2). Each farrowing crate was equipped with a feeder and a nipple waterer and a heat lamp was available for newborn piglets. Within 24 h of farrowing, Fe-dextran (150ppm) injection, notching ears, clipping teeth and tail were done. The weaning to estrous interval of multiparous sows was checked from 3 to 12 d after weaning.

Experimental Diets

The experimental diets contained different energy levels (3,165 or 3,265 kcal of ME/kg) and NSP enzyme supplementation levels (0 or 0.1%) in gestation period. NSP enzyme have specific activities (α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea, Table 1). Then, all other nutrients were met or exceeded the requirements of NRC (1998), and sows were fed 2.4 kg of diet daily during gestation. During lactation, the same diet was provided *ad libitum* regardless of dietary treatments with a free access to water. The formulas and chemical composition of experimental diets in gestation and lactation periods were presented in Tables 2 and 3.

Physiological Responses of Sows and Piglets

The BW and backfat thickness of multiparous sows were measured at 0 and 110 d postmating and 24 h and 21 d postpartum. Individual piglet weight and litter size of lactating sows were also checked at 0 h and 21 d postpartum and the weaning to estrus interval (WEI) of sows was recorded after weaning. Voluntary feed intake of sows was measured during lactation period.

Blood Profiles

The blood samples of multiparous sows were collected in EDTA tubes during gestation (0, 15, 35, 70, 90 and 110 d postmating) and lactation period (24 h, 7, 14 and 21 d postpartum). Individual sample was centrifuged at 3,000 rpm on 4 °C for 15 min, and then plasma was separated from blood samples and kept at -20 °C until analysis. The concentration of estradiol and insulin were analyzed and content of serum glucose was detected by an enzymatic kit (Glucose Hexokinase Kit, Bayer, USA). The concentration of progesterone in the peripheral blood plasma was determined using a solid-phase RIA kit (Coat-A-Count® Progesterone; Diagnostic Products Corporation, Los Angeles, CA, USA).

Proximate Analysis of Colostrum and Milk

Colostrum (24 h postpartum) and milk of sows at 7, 14 and 21 d postpartum were collected from the first and second teats after 5 IU oxytocin injection (Komi oxytocin inj. Komipharm international Co., Ltd., Korea) and collected milk samples were stored at -20 °C until analysis. Proximate analysis of colostrum and milk was conducted using Milkoscan FT 120 (FOSS Electric).

Body Condition Scoring

Body condition score (BCS) of sows was measured during gestation (0, 15, 35, 70, 90 and 110 d postmating). This scoring system was done by finger or hand pressure at key points on the sow's body. The points used on the sow's body are those areas where the only tissue between the skin and bones is fat tissue. These areas on the sow include the ribs, back bone, "H" bones, and "pin" bones (Figure 1). By assessing the ease or difficulty of feeling these bones, the fat stores of the sow was estimated by 1 to 5 score.

Statistical Analysis

All of collected data were carried out by least squares mean comparisons and were evaluated using PDIFF option with the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). Individual sows and their litters were used as the experimental unit and were analyzed as a 2 x 2 factorial arrangement, and differences were declared significant at $P<0.05$ and highly significant at $P<0.01$.

RESULTS

The effects of dietary energy levels and enzyme supplementation in gestation diet on body weight, back-fat thickness and voluntary feed intake of sows were shown in Table 4. During the whole experimental period, there was no significant difference in the results of body weight and backfat thickness during gestation. However, the weight gain from 0 to 110 d of gestation tended to increase when the sows fed diet containing 3,265 kcal of ME/kg (energy response, $P=0.09$). The sows fed diet containing NSP enzyme showed numerical increase of weight gain (47.4 kg and 51.65 kg, respectively). Although the significant effects were not found in body weight, backfat thickness and ADFI during lactation, the sows fed diets containing NSP enzyme showed numerically decrease in body weight change and voluntary feed intake during lactation (0-21 d). Then, this numerical difference was increased

when sows were fed diet containing 3,165 kcal of ME/kg relative to those fed diet containing 3,265 kcal of ME/kg. In the results of reproductive performance and litter growth, the average weight of piglets at birth was increased when the sows fed high energy (energy response, $P<0.01$) and enzyme diets (enzyme response, $P<0.05$). When sows were fed diet containing 3,265 kcal of ME/kg with 0.1% NSP enzyme showed significantly higher piglet weight at birth, involved in small total born size. (Table 5).

The effects of dietary energy levels and enzyme supplementation in gestation diet on the plasma concentrations of estradiol, progesterone, insulin and glucose were presented in Tables 6 and 7. The significant responses of dietary treatments were not detected in blood profiles except for the concentrations of progesterone at mating, insulin at 70 d of gestation and glucose at 110 d of gestation. Increased plasma concentrations of progesterone at mating and insulin at 70 d were observed when NSP enzyme was added to gestation diet (enzyme response, $P<0.05$), and decreased plasma concentration of glucose at 110 d was detected when dietary energy level increased (energy response, $P<0.05$). Although there was no significant difference, the plasma concentrations of progesterone at 35 d of gestation tended to decrease as dietary energy level increased ($P=0.07$), and that of insulin at 21 d of lactation also tended to decrease when enzyme was added to gestation diet ($P=0.09$). But there were no differences in hormone characteristics during lactating(Table 7).

The colostrum and milk composition of lactation including milk fat, protein, total solid and solids-not-fat were not significantly differed as dietary treatments were changed (Table 8). The content of lactose at 7 d of lactation was increased when NSP enzyme was added to gestation diet (enzyme response, $P<0.05$). Even though significant interaction between two main factors was not detected, the content of milk solid not fat at 14 d of lactation ($P=0.05$) and BCS of sows at 90 d

and 110 d of gestation ($P<0.10$) tended to be affected by this interaction (Figure 2).

DISCUSSION

Verstegen et al. (1987) and Noblet et al. (1990) reported that the weight gains of fetus and conceptus were increased by about 20 kg and the weight gain of sow was increased by about 45 kg in gestation. In the current study, the average weight gain of gestating sows was 50 kg, and the sows fed containing low energy diets showed higher response of enzyme supplementation compared to those fed containing high energy diets. According to Charlton (1996) and Schang et al. (1997), the response of enzyme supplementation in gestation diet was not detected when dietary nutrient levels were met or exceeded the requirements of animals, and these findings might be an explanation for higher NSP enzyme response of sows fed low energy diets in this study. Backfat thickness of gestating sows could be an indicator of energy accumulation, and was elevated as dietary energy intake increased. In the result of the present study, the sows fed diet with NSP enzyme showed numerically higher backfat gains than those fed diet without NSP enzyme. Frobish (1970) suggested that increasing dietary energy level could induce increased fat accumulation and decreased voluntary feed intake in lactation, and Reesem et al. (1982) and Trottier and Johnston (2001) reported that voluntary feed intake was closely associated with body weight losses in lactation, and insufficient energy intake during lactation had negative influence on subsequent reproductive performance because sows mobilized their body reserves for producing milk. In the current study, the voluntary feed intake during lactation was not changed by dietary treatments. Thus, it was expected that growth performance of nursery piglets and the changes of body weight and backfat thickness of lactating sows also were not affected by dietary treatments.

Decreased concentration of serum progesterone and implantation rate of

embryo in sows fed high energy diets during early gestation have been demonstrated many times in previous studies (Dyck and Strain, 1983; Toplis et al., 1983; Jindal et al., 1996). When sows were fed high energy diets with NSP enzyme, the concentration of serum progesterone at early gestation and the number of total born after farrowing were decreased numerically. Then, this result may be due to the fact of excess energy intake or increased energy digestibility by the supplementation of NSP enzyme. Generally, it is well known that the parity, the number of total born, body weight of piglets at birth and body shape of sows had influenced on the number of stillbirth (Leenhouwers et al., 1999; Lucia et al., 2002; Schneider, 2002), but there was no significant difference in the number of stillbirth in this study. There were few findings reporting the positive correlation between dietary energy intake during gestation and litter weight at birth (NRC, 1998), but dietary energy levels of these findings were relatively lower than those of other findings reporting no significant difference of these dietary treatments. The results of this study indicated that dietary energy level had no influence on litter weight at birth when dietary energy level meets or exceeds 3,165 kcal of ME/kg. In the present study, average piglet weight at birth was increased when sows fed diets containing high level of energy and NSP enzyme ($P<0.05$), but this response may be due to very high piglet weight at birth of high energy and enzyme-supplement treatment, derived from very low litter size.

Many findings demonstrated that embryo development was affected by the blood concentration of estradiol, and the concentration of this hormone was elevated as pregnancy day increased (Tucker et al., 1994). Then, Foisnet et al. (2008) reported that estradiol was associated with lactogenesis, and improved production of colostrum was observed when the blood concentration of estradiol increased in gestation. Even though there was no detectable effect of dietary treatments, the blood concentration of estradiol in sows fed diets with NSP enzyme

was numerically higher than those fed diets without NSP enzyme during the whole gestation period. Jindal et al. (1997) suggested that the blood concentration of progesterone was decreased when gestating sows fed high energy diets, and the result of the present study was agreed with this study, wherein the plasma concentrations of progesterone at 35 d of gestation tended to be reduced as dietary energy level increased ($P=0.07$). There were many findings suggesting a positive correlation between energy intake and blood concentration of insulin (Flowers et al., 1989), agreed with this study, wherein the plasma concentrations of insulin at 70 d was increased when NSP enzyme was added to gestation diet ($P<0.05$). In addition, no effects were found in the blood concentrations of estradiol, progesterone, insulin and glucose in lactation. These results demonstrated the fact that control diet was provided to sows during lactation regardless of dietary treatments during gestation.

Milk composition of sows is affected by the species, body condition and dietary nutrient intake, and the development of mammary gland progresses from 75 to 90 d of gestation (NRC, 1998). Therefore, excess energy intake during this stage could reduce the number of mammary alveolar cell, resulting in decreased milk production of lactating sows (Weldon et al., 1994). In the current study, although the enzyme response on the content of lactose at 7 day ($P<0.05$) and interaction effect on the content of solid not fat at 14 day ($P=0.05$) were observed for milk components of lactating sows, other parameters were not affected by dietary treatments. These results are agreed with Williams (1995) who found that the chemical composition of milk was not affected by dietary nutrient level because lactating sows mobilized their body reserves to support the deficient nutrients.

Applying body condition score (BCS) to evaluate the condition of sows has some problems such as a lack of objectivity, low accuracy and high variation in modern sows, however this parameter has been used widely in swine industry because of convenience (Kim et al., 2011). In the present study, BCS was not

significantly changed by dietary treatments, but BCS of sows at 90 d and 110 d of gestation ($P<0.10$) tended to be affected by the interaction between dietary energy levels and enzyme supplementation, resulting in improved BCS by the supplementation of NSP enzyme when the sows fed low energy diets. This result of BCS is agreed with that of weight gain in gestation, and might be explained by same mechanism. Long(2010) found out that body weight gain at 3rd parity gestating sow was elevated when sows were fed high energy diet and the point of inflection was observed around 3,265 kcal of ME/kg.

CONCLUSION

To achieve successful gestation and subsequent high productivity of sow, the effects of NSP enzyme on physiological responses and reproductive performance of sows were evaluated. When sows were fed diet containing NSP enzyme showed numerically increased weight gain from 0 to 110 d of gestation, and decreased loss of body weight and voluntary feed intake in lactation (0-21 d), although the body weight, backfat thickness, and ADFI in lactation were not changed by dietary treatments. This numerical difference was increased when the sows fed diet containing 3,165 kcal of ME/kg relative to those fed diet containing 3,265 kcal of ME/kg. The sows fed high energy (energy response, $P<0.01$) or enzyme diets (enzyme response, $P<0.03$) showed increased average weight of piglets at birth. Consequently, when sows were fed diet containing 0.10% NSP enzymes regardless of energy levels showed beneficial effects on body weight, back fat thickness change of sows, and the effects of supplementing NSP enzyme were greater when low energy diet was provided.

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Table 1. Specific activities of NSP enzyme

Specific enzyme	Activity
α-galactosidase	800 unit[1)]/g
galactomannanase	2,500 unit[2)]/g
xylanase	3,500 unit[3)]/g
β-glucanase	2,500 unit[3)]/g

[1)] Unit is equal to 0.01 μ mol pNPG hydrolyzed within 1 min. at 30℃ and pH 4.0.

[2)] Unit is equal to 0.01mg total reducing sugar-glucose equivalent-released within 1min. at 40℃ and pH 4.0.

[3)] Unit is equal to 0.1 mg total reducing sugars-glucose equivalent-released within 1 min. at 30℃ and pH 4.0.

Table 2. The formulas and chemical composition of gestation diet

Treatments	ME, kcal/kg	3,165		3,265	
	Enzyme, %	0	0.1	0	0.1
Ingredients, %					
Yellow corn		61.81	61.71	58.83	58.73
Wheat bran		7.46	7.46	8.06	8.06
Rice bran		5.00	5.00	5.00	5.00
Beet pulp		4.00	4.00	4.00	4.00
Soybean meal		12.38	12.38	12.62	12.62
Palm kernel meal		5.00	5.00	5.00	5.00
Soy oil		1.62	1.62	3.76	3.76
Choline-Cl		0.09	0.09	0.09	0.09
TCP		1.34	1.34	1.34	1.34
Limestone		0.60	0.60	0.60	0.60
Salt		0.50	0.50	0.50	0.50
Vit. mix[1)]		0.10	0.10	0.10	0.10
Min. mix[2)]		0.10	0.10	0.10	0.10
Multi Enzyme[3)]		-	0.10	-	0.10
Total		100.00	100.00	100.00	100.00
Chemical composition [4)]					
CP, %		13.00	13.00	13.00	13.00
C.Fat, %		5.33	5.33	7.38	7.38
C.Fiber		4.50	4.50	4.50	4.50
C.Ash, %		5.21	5.21	5.22	5.22
Lys, %		0.62	0.62	0.62	0.62
Ca, %		0.75	0.75	0.75	0.75
Total P, %		0.67	0.67	0.67	0.67

[1)] Provided per kg of diet : Vitamin A, 12,000 IU; Vitamin D_3, 2,400 IU; Vitamin E, 100 mg; Vitamin K_3, 3 mg; Vitamin B_1, 3 mg; Vitamin B_2, 8 mg; Niacine, 30 mg; Folic acid,4 mg; Vitamin B_{12}, 60 mg.
[2)] Provided per kg of diet : Mn, 80 mg; Zn, 30 mg; Fe, 150 mg; Cu, 5 mg; Co, 0.5 mg;
[3)] Multi enzyme : Endo-Power ® (α-galactosidase + galactomannanase + xylanase + β-glucanase; EASY BIO, Inc., Korea)
[4)] Calculated value.

Table 3. The formulas and chemical composition of lactation diet[1)]

Ingredients, %	Lactating diet
Yellow corn	55.43
Wheat bran	5.00
Soybean meal	28.98
Palm kernel meal	5.00
Tallow	2.67
L-Lysine HCl	0.02
DCP	1.28
Limestone	0.92
Salt	0.50
Vit. Mix.[2)]	0.10
Min. Mix.[3)]	0.10
Total	100.00
Chemical composition[4)]	
ME, kcal/kg	3,265.0
CP, %	18.40
C.Fat, %	5.52
C.Fiber, %	4.14
C.Ash, %	5.41
Lys, %	0.98
Ca, %	0.75
Total P, %	0.63

[1)]Lactation diet was provided *ad libitum*.
[2)] Provided per kg of diet : Vitamin A, 12,000 IU; Vitamin D_3, 2,400 IU; Vitamin E, 100 mg; Vitamin K_3, 3 mg; Vitamin B_1, 3 mg; Vitamin B_2, 8 mg; Niacine, 30 mg; Folic acid,4 mg; Vitamin B_{12}, 60 mg.
[3)] Provided per kg of diet : Mn, 80 mg; Zn, 30 mg; Fe, 150 mg; Cu, 5 mg; Co, 0.5 mg;
[4)] Calculated value.

Table 4. Effects of dietary energy levels and NSP enzyme supplementation in gestation diet on body weight and backfat thickness of sows

Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Enzyme, %	0	0.1	0	0.1		Eng	Enz	ExE
No. Sows		8	8	11	10				
Body weight, kg									
Gestation									
At mating		225.3	228.9	217.3	215.2	3.23	0.35	0.88	0.80
110 days of gestation		269.6	278.8	267.8	268.6	3.08	0.77	0.63	0.63
Gain (0-110 d)		44.3	49.9	50.5	53.4	3.87	0.09	0.32	0.80
Lactation									
24 hrs postpartum		258.1	256.6	254.6	250.9	3.45	0.83	0.95	0.24
21 days of lactation		252.9	256.4	247.1	244.8	3.45	0.92	0.53	0.23
Changes (0-21 d)		-5.1	-0.2	-7.5	-6.1	2.00	0.70	0.25	0.56
Back-fat thickness, mm									
Gestation									
At mating		19.3	20.0	19.2	18.6	0.74	0.96	0.90	0.91
110 days of gestation		22.6	23.9	22.6	22.3	0.81	0.78	0.83	0.77
Gain (0-110 d)		3.3	3.9	3.4	3.8	0.35	0.44	0.86	0.64
Lactation									
24 hrs postpartum		23.7	23.5	23.1	21.3	0.89	0.54	0.23	0.60
21 days of lactation		22.4	22.8	21.5	20.2	0.91	0.58	0.40	0.52
Changes (0-21 d)		-1.3	-0.7	-1.6	-1.1	0.18	0.85	0.23	0.49
ADFI in lactation, kg		6.07	5.78	6.09	5.85	1.149	0.84	0.34	0.96

[1)] Standard error of mean.

Table 5. Effects of dietary energy levels and enzyme supplementation in gestation diet on reproductive performance of sows

Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Enzyme, %	0	0.1	0	0.1		Eng	Enz	ExE
No. Sows		7	8	9	9				
Litter size, no. of piglets									
Total born		13.14	13.75	13.11	10.22	0.613	0.14	0.34	0.15
Still born		0.57	1.13	0.56	0.11	0.209	0.23	0.90	0.24
Mummy		0.86	0.63	1.00	1.22	0.317	0.58	0.99	0.74
Born alive		11.71	12.00	11.56	9.00	0.629	0.22	0.37	0.26
After cross-fostering		10.57	11.25	10.78	9.56	0.200	0.15	0.34	0.24
Weaning pigs		10.14	10.75	10.56	9.22	0.190	0.13	0.13	0.23
Mortality		3.90	4.55	2.02	3.03	0.956	0.48	0.58	0.77
Litter weight, kg									
At birth		19.06	19.02	18.74	18.58	0.473	0.70	0.91	0.94
After cross-fostering		15.53	16.90	16.59	17.11	0.431	0.48	0.27	0.63
21d		59.68	57.60	61.73	56.91	1.456	0.82	0.26	0.65
Weight gain (0-21d)		44.15	40.70	45.14	39.80	3.377	0.99	0.09	0.72
Piglet weight, kg									
At birth		1.38	1.43	1.47	1.83	0.050	<0.01	0.03	0.06
After cross-fostering		1.51	1.50	1.54	1.78	0.041	0.48	0.29	0.63
21d		6.00	5.38	5.86	6.14	0.155	0.82	0.26	0.65
Weight gain (0-21d)		4.49	3.88	4.32	4.36	0.352	0.57	0.30	0.23

[1)] Standard error of mean.

Table 6. Effects of dietary energy levels and enzyme supplementation in gestation diet on blood profiles of gestating sows

Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Enzyme, %	0	0.1	0	0.1		Eng	Enz	ExE
Estradiol, pg/mL									
Initial		40.4	62.6	49.2	56.4	4.117	0.99	0.95	0.99
15 day		41.7	46.2	42.5	53.8	3.470	0.56	0.27	0.64
35 day		47.1	56.1	48.0	58.6	4.011	0.85	0.23	0.97
70 day		59.8	80.0	75.0	86.2	7.248	0.42	0.16	0.68
90 day		206.4	244.3	199.9	254.8	17.241	0.96	0.20	0.81
110 day		426.5	484.1	446.6	538.8	24.828	0.46	0.73	0.73
Progesterone, ng/mL									
Initial		0.5	0.7	0.5	0.8	0.048	0.51	0.03	0.26
15 day		50.6	53.4	46.0	48.5	2.163	0.29	0.56	0.98
35 day		39.8	39.7	35.7	31.4	1.688	0.07	0.51	0.52
70 day		34.7	33.2	36.1	30.9	1.358	0.87	0.23	0.52
90 day		31.7	28.6	30.2	33.0	1.577	0.66	0.98	0.37
110 day		24.6	25.1	25.2	24.6	1.215	0.86	0.94	0.82
Insulin, μU/mL									
Initial		1.1	1.3	1.1	1.4	0.116	0.57	0.26	0.88
15 day		0.5	0.5	0.8	0.6	0.092	0.43	0.60	0.67
35 day		0.6	0.8	0.6	0.7	0.054	0.59	0.33	0.56
70 day		0.6	0.8	0.5	0.7	0.053	0.35	0.04	1.00
90 day		0.7	0.8	0.6	0.7	0.070	0.18	0.25	0.98
110 day		0.6	0.7	0.7	0.5	0.051	0.67	0.56	0.26
Glucose, mg/dL									
Initial		78.5	72.3	72.2	75.3	2.216	0.67	0.71	0.32
15 day		62.6	56.4	56.0	59.2	2.068	0.66	0.72	0.27
35 day		62.6	61.8	65.8	63.3	1.425	0.43	0.57	0.78
70 day		66.9	69.8	66.8	67.2	0.994	0.59	0.37	0.57
90 day		70.6	68.9	73.5	70.2	0.935	0.28	0.19	0.69
110 day		75.8	73.9	69.8	68.3	1.481	0.05	0.57	0.94

[1)] Standard error of mean.

Table 7. Effects of dietary energy levels and enzyme supplementation in gestation diet on blood profiles of lactating sows

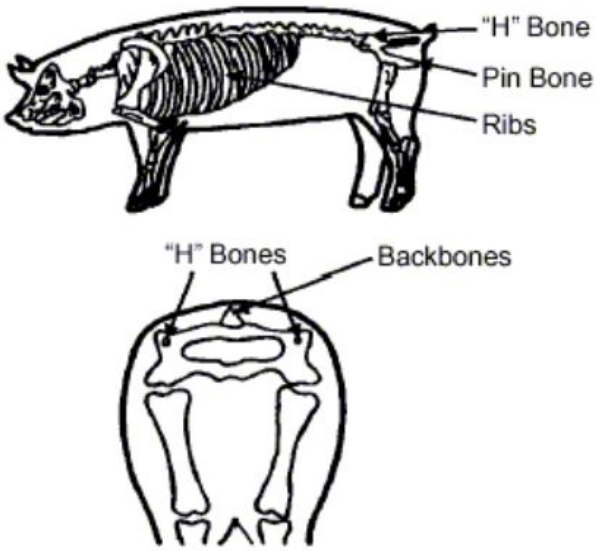
Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Enzyme, %	0	0.1	0	0.1		Eng	Enz	ExE
Estradiol, pg/mL									
Initial		136.6	119.7	130.5	197.5	15.33	0.24	0.41	0.17
7 day		36.0	44.3	39.5	53.5	4.53	0.50	0.24	0.76
14 day		36.6	39.4	31.9	45.0	3.36	0.95	0.25	0.46
21 day		26.6	31.8	33.1	34.7	3.16	0.50	0.64	0.79
Progesterone, ng/Ml									
Initial		4.7	5.1	3.9	4.6	0.36	0.45	0.43	0.84
7 day		0.5	0.5	0.4	0.6	0.05	0.89	0.58	0.24
14 day		0.4	0.4	0.4	0.4	0.03	0.73	0.84	0.59
21 day		0.4	0.4	0.4	0.4	0.03	0.90	0.26	0.84
Insulin, μU/mL									
Initial		2.1	1.4	3.1	2.3	0.29	0.30	0.23	0.69
7 day		3.9	3.4	3.2	1.9	0.40	0.19	0.24	0.63
14 day		1.9	2.5	2.1	2.3	0.18	0.96	0.30	0.62
21 day		2.6	2.2	3.4	2.0	0.25	0.58	0.09	0.35
Glucose, mg/dL									
Initial		91.3	81.0	89.8	89.1	2.29	0.50	0.33	0.32
7 day		87.7	83.7	84.6	83.1	1.29	0.50	0.32	0.65
14 day		80.7	85.3	86.8	79.8	1.63	0.93	0.71	0.09
21 day		79.6	81.9	82.1	80.4	1.43	0.86	0.92	0.53

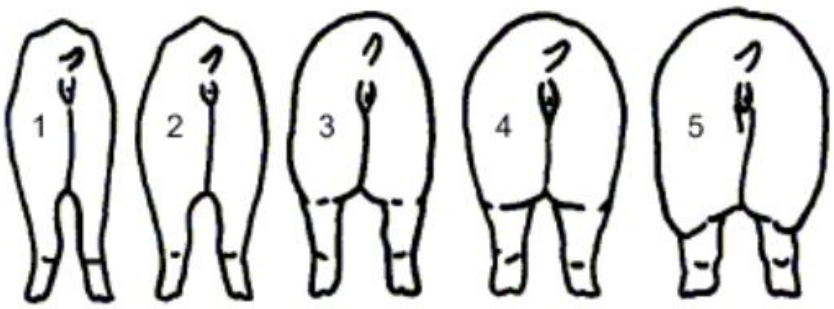
[1)] Standard error of mean.

Table 8. Effects of dietary energy levels and enzyme supplementation in gestation diet on colostrum and milk components of sows

Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Energy, %	0	0.1	0	0.1		Eng	Enz	ExE
Fat, %									
Colostrum		5.91	6.73	6.07	6.07	0.287	0.69	0.49	0.51
7 d		6.75	6.90	6.93	7.51	0.140	0.15	0.19	0.47
14 d		6.57	6.75	6.74	6.64	0.118	0.90	0.86	0.58
21 d		6.86	6.79	6.53	6.60	0.133	0.41	0.98	0.80
Protein, %									
Colostrum		6.94	6.13	6.97	8.03	0.339	0.17	0.87	0.17
7 d		4.75	4.77	4.75	4.55	0.045	0.26	0.37	0.20
14 d		4.51	4.67	4.73	4.56	0.046	0.58	0.91	0.13
21 d		4.84	4.87	4.81	4.77	0.046	0.54	0.91	0.82
Lactose, %									
Colostrum		4.33	4.50	4.28	4.16	0.062	0.15	0.89	0.22
7 d		5.66	5.76	5.66	5.77	0.024	0.91	0.04	0.89
14 d		5.92	5.87	5.82	5.88	0.024	0.36	0.82	0.21
21 d		5.82	5.88	5.88	5.83	0.020	0.87	0.79	0.28
Total solid, %									
Colostrum		19.97	19.18	20.12	19.88	0.511	0.70	0.64	0.81
7 d		18.61	18.78	18.69	19.22	0.172	0.49	0.34	0.61
14 d		18.51	18.62	18.57	18.57	0.124	0.97	0.80	0.82
21 d		18.97	18.88	18.44	18.54	0.170	0.23	0.99	0.81
Solid not fat, %									
Colostrum		11.65	10.96	11.54	12.05	0.245	0.34	0.83	0.25
7 d		10.62	10.66	10.62	10.46	0.035	0.15	0.35	0.22
14 d		10.55	10.65	10.67	10.53	0.035	0.98	0.67	0.05
21 d		10.86	10.86	10.81	10.80	0.037	0.37	0.87	0.91

[1)] Standard error of mean.





Score	Condition	Detection of bone
1	Emaciated	Obvious
2	Thin	Easy detected with pressure
3	Ideal	Barely felt with firm pressure
4	Fat	None
5	Overly fat	None

Figure 1. Body condition score (BCS) of sow and scoring description (Richard et al, 2000)

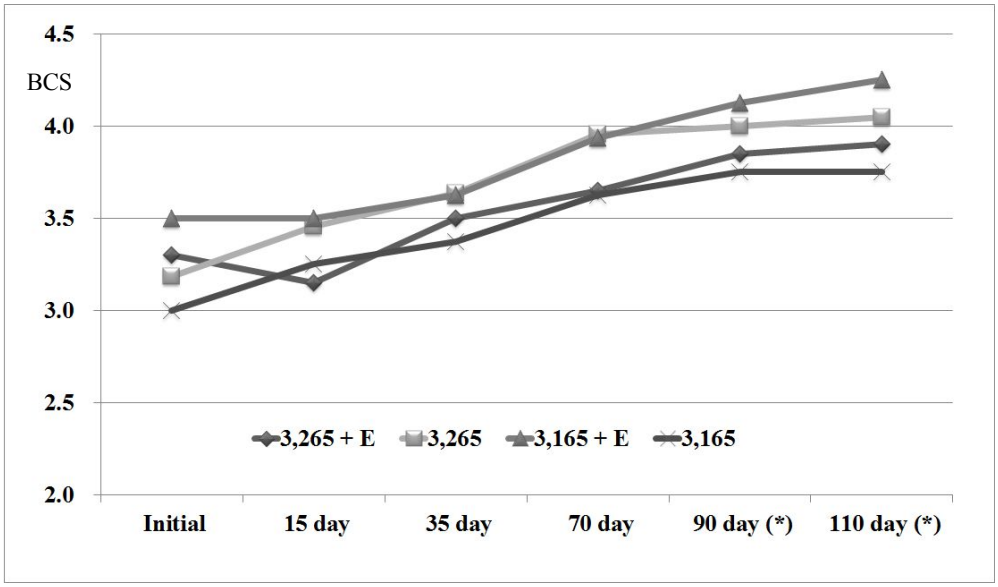


Figure 2. Effects of dietary energy levels and enzyme supplementation in gestation diet on body condition score of sows (*: interaction effect between dietary enzyme and energy levels, P<0.1)

Chapter IV: Effects of Energy Modulation and NSP Enzyme Supplementation in Lactation Diet on Physiological Responses and Reproductive Performance of Lactating Sows

ABSTRACT: A study was conducted to evaluate the effect of dietary energy levels and NSP enzyme supplementation in lactation diet on physiological response and reproductive performance of lactating sows. A total of 40 mixed-parity sows (F1, Yorkshire x Landrace, 5.8±0.8 parity; Darby, Korea) with an initial BW of 249.66 ± 8.86 kg were used for a trial, and were allotted to one of four treatments based on BW and backfat thickness in a completely randomized design (CRD) with a 2 x 2 factorial arrangement after farrowing. The first factor was energy level in diets (3,165 or 3,265 kcal of ME/kg), and the second factor was NSP enzyme complex inclusion (α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea). The experimental diets were formulated based on corn and soybean meal and contained 18.98 or 18.88% crude protein, 0.99% lysine, 0.75% Ca, and 0.64% total P, and all other nutrients were met or exceeded the requirements of NRC (1998). During lactation, experimental diets were provided *ad libitum* regardless of dietary treatments with a free access to water. The body weight change of sows was not affected by dietary treatments and numerical reduction of body weight loss from 0 to 21 d of lactation was observed in sows fed diet contained 3,265 kcal of ME/kg compared with those fed diet contained 3,165 kcal of ME/kg in lactation (P=0.09). Interaction effect between dietary energy level and enzyme supplementation was observed in the result of backfat thickness at 21 d postpartum (interaction effect, P<0.05), and WEI of sows was not differed among treatments after weaning. However, ADFI in lactation was decreased when sows were fed high energy (energy response, P<0.01) and enzyme

diets (enzyme response, $P<0.01$). Feeding diets containing 3,265 ME kcal/kg or 0.10% NSP enzyme had no effects on litter size and litter performance of lactating sows except for the average weight gain of piglets from 0 to 21 d of lactation (energy response, $P<0.05$), and the contents of fat, protein, total solid and solids-not-fat in colostrum and milk also were not changed by dietary treatments. The content of milk lactose at 21 d of lactation tended to be increased when sows were fed diet containing 3,265 kcal of ME/kg compared with those fed diet containing 3,165 kcal of ME/kg ($P=0.09$). Consequently, when sows were fed low energy diet (3,165 kcal of ME/kg) with dietary NSP enzyme, they showed similar physiological responses and litter performance of lactating sows compared to those fed high energy diet (3,265 kcal of ME/kg).

Key words: NSP Enzyme, Lactating Sow, Suckling Pig, Litter Performance

INTRODUCTION

In general, the most commonly used feed ingredients in swine feed are grains and their residues. For instances, corn, wheat and barley are widely used as energy source, whereas soybean meal and rapeseed meal are major protein sources. Although these feed ingredients had better nutritional composition and bioavailability than other feed ingredients, many nutritionists have conducted experiments for improving feed availability by feed processing or feed additive. With this research background, international grain prices including major feed ingredients have been increased dramatically because of severe drought both in U.S. and Europe or production of biofuel. So, it was needed to improve availability of major feed ingredients or to find alternative feed ingredients by using dietary enzymes. Although searches and development of alternative ingredient have been accelerated, these alternative materials were underestimated compared to conventional feed ingredients, because most alternative ingredients contained the high contents of anti-nutritional factors.

The major component of anti-nutritional factors is Non Starch Polysaccharide (NSP), and it is a non-digestible carbohydrate and may inhibit availability of other nutrients. Dietary level of NSP is associated with reducing digestibility of mono-gastric animal without NSP enzymes to break this nutrient, subsequently various experiments about NSP enzymes were conducted. In the present research, representative NSP enzymes contain α-galactosidase, galactomannanase, xylanase and β-glucanase. Their modes of actions are summarized in four mechanisms. At first, decreasing NSP has influences on secretion of insulin and absorption of glucose for energy metabolism. Secondly, decreasing NSP reduced the viscosity of digesta and improved the activity of absorption as well as digestion in gastro-intestinal tract (Dale, 1997). Thirdly, degraded oligosaccharide from NSP had positive effect on intestinal

mircroorganisms. Lastly, mannan, one of major NSP, is converted to mannan oligosaccharide, resulting in activating immune response. Comprehensively, supplementation of NSP enzyme in feed is expected to improve energy utilization by increasing the nutrient digestibility and glucose availability, especially supplementation of NSP enzyme in low energy diets has complementary effect on energy balance. It is well known that body weight of lactating sows is decreased during lactation because the required energy for recovering body condition and producing milk is greater than dietary energy intake (Mullan and Williams, 1989; Yang et al., 1989). Then, improving the nutrient availability by degrading the anti-nutritional factors is essential to reduce the body weight loss in lactating sow, and the supplementation of NSP enzyme may have positive effect on longevity of sows and piglet performance.

Therefore, this study was conducted to investigate effect of NSP enzyme (Endo-power□; galactosidase, galactomannanase, xylanase and β-glucanase) supplementation on physiological responses and reproductive performance of lactating sows.

MATERIALS AND METHODS

Animal and Housing

A total of 40 mixed-parity sows (F1, Yorkshire x Landrace; 5.8±0.8 parity; Darby, Korea) with an initial body weight of 249.66 ± 8.86 kg were used for a trial, at a research farm located in Eum-seong, Korea. Sows were allotted to one of four treatments based on BW and backfat thickness in a completely randomized design (CRD). On 110 d of gestation, sows were moved into environmentally controlled farrowing rooms and placed in individual farrowing crate (2.5 x 1.8 m^2). Each farrowing crate was equipped with a feeder and a nipple waterer for sow and a heat lamp for newborn piglets. Dietary treatments were assigned to the sows after farrowing, and then the BW and backfat thickness of sows were measured at 24 h

postpartum and 21 d of lactation. Individual piglet weight and litter size of sows were checked at same time, and then the weaning to estrus interval (WEI) of sows was recorded after weaning as a important parameter for evaluating reproductive performance.

Experimental Diets and Treatments

The experimental diets containing different energy levels (3,165 or 3,265 kcal of ME/kg) with or without supplementation of 0.1% NSP enzyme (α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea) was supplied in the lactation period, diet contained 18.98% or 18.88% crude protein, 0.99% lysine, 0.75% methionine and 0.64% total phosphorous respectively. All other nutrients met or exceeded the requirements of NRC (1998) and feed was provided *ad libitum* with a free access to water. The chemical composition of experimental diets is presented in Table 1.

Physiological Responses of Sows and Piglets

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

Proximate Analysis of Colostrum and Milk

Colostrum (24 h postpartum) and milk of sows at 21 d postpartum were collected from the first and second teats after 5 IU oxytocin injection (Komi oxytocin inj. Komipharm international Co., Ltd., Korea) and collected milk samples were stored at -20 °C for later analysis. Proximate analysis of colostrum and milk

was conducted using Milkoscan FT 120 (FOSS Electric).

Statistical Analysis

All of collected data were carried out by least squares mean comparisons and were evaluated using PDIFF option with the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). Individual sows and their litters were used as the experimental unit and were analyzed as a 2 x 2 factorial arrangement, and differences were declared significant at $P<0.05$ and highly significant at $P<0.01$.

RESULTS

The effects of dietary energy level and NSP enzyme supplementation in lactating diet on the body weight, backfat thickness, WEI and average voluntary feed intake of lactating sows were presented in Table 2. The body weight and body weight changes were not significantly affected by dietary treatments, but the losses of body weight from 0 to 21 d of lactation tended to be decreased in sows fed diet contained 3,265 kcal of ME/kg compared to those fed diet contained 3,165 kcal of ME/kg in lactation ($P=0.09$). WEI of sows was not different among treatments, but ADFI in lactation was decreased when the sows fed high energy (energy response, $P<0.01$) or enzyme diets (enzyme response, $P<0.01$). In the results of litter size and litter performance, there was no significant difference except for the average weight gain of piglets from 0 to 21 d of lactation (energy response, $P<0.05$), and the contents of fat, protein, total solid and solids-not-fat in colostrum and milk also were not affected by dietary treatments. However, the content of milk lactose at 21 d of lactation tended to be increased when the sows fed diet containing 3,265 kcal of ME/kg compared with those fed diet containing 3,165 kcal of ME/kg ($P=0.09$).

DISCUSSION

Supplementing NSP enzyme to lactation diet had no effects on the changes of body weight in lactating sows (0-21 d), but this parameter tended to be decreased when sows were fed high energy diets (P=0.09) with a numerical decrease when NSP enzyme was added to lactation diet. In general, body weight of sows after weaning is lower than that after farrowing, because dietary nutrient intake of lactating sows is not enough to produce milk regardless of the amounts of voluntary feed intake (Mullan and Williams, 1989; Yang et al., 1989), these changes agreed with the result of current study. There is a lack of information on the use of NSP enzymes in lactation diet for sows because the lactating sows have higher fiber digestibility than that of growing-finishing pigs (Adeola and Cowieson, 2011). Then, Souza et al. (2007) demonstrated that total tract DM and N digestibilities of lactating sows were improved when protease and xylanase were added to lactation diet. In the present study, although the digestibility of lactating sows was not analyzed during lactation, the loss of body weight was reduced numerically when NSP enzyme was added to lactation diet, which could be explained by the results of Souza et al. (2007).

During the whole experimental period, the sows fed high energy or NSP enzyme diets showed decreased voluntary feed intake compared with those fed low energy or no NSP enzyme diets, respectively (energy and enzyme response; P<0.01). Dourmad et al. (1991) reported that decreased voluntary feed intake of sows was observed as dietary energy level increased during lactation, and there were many findings supporting this result. However, there was no reference related to the effects of dietary NSP enzymes on voluntary feed intake of lactating sows. Many findings suggested that carbohydrase supplementation had a positive effect on energy digestibility of pigs (Yin et al., 2000; Diebold et al., 2004; Olukosi et al., 2007), and this difference of energy intake might cause decreased feed intake of

lactating sows in the present study. Possible explanations could be affected for detectable effects of the NSP enzyme on feed consumption of lactating sows, and further study is needed to demonstrate this effect clearly. King and Williams (1984) and Baidoo et al. (1992) reported that decreasing feed consumption lead to delay weaning to estrus interval (WEI), but dietary energy levels and supplementation of NSP enzyme in lactating sows did not affect WEI in this study.

During the whole experimental period, there was no significant difference on litter size and performance except for average weight gain of piglets from 0 to 21 d of lactation (energy response, $P<0.05$). Clowes et al. (1988) demonstrated that average weight of piglets was related to the amounts of milk production, and 1 g weight gain of nursery piglets was associated with 3.88 g of milk consumed, Noblet and Etienne (1989) also reported that milk production of sows was critical to the growth rate of piglets. Even though the milk yield of this study was not measured, numerically increased contents of lactose and fat were detected in milk of sows fed high energy diets. Then, this difference also could be a reason of energy response in the result of average weight gain of piglets. In the current study, milk composition of sows was not affected by dietary treatments, but the content of lactose in milk was decreased when low energy diet (3,165 kcal of ME/kg) was provided ($P=0.09$). Insufficient feed intake resulted in reduced weight, decreased backfat thickness, and leg bone weakening, because the body reserves of sows were used for milk production regardless of the amounts of dietary nutrient intake, resulting in unchanged milk composition (Dourmad et al., 1994). Although there was a trend in the contents of lactose at 21 d of lactation, most nutrients in milk were not changed by dietary treatments, which are agreed with previous findings.

In the present study, the effect of enzyme on backfat thickness of sows at 21 d of lactation was thicker in sows fed low energy diets relative to those fed high energy diets (interaction effect, $P<0.05$). These data are agreed with Charlton (1996)

and Schang et al. (1997) who demonstrated that the response of enzyme supplementation was changed as dietary energy level increased because of nutrient requirements of pigs.

CONCLUSION

This study showed high energy or enzyme supplementation diet improved lactating sow performance in aspects of sow weight loss, litter weight gain and milk composition during lactation. And enzyme supplementation had more beneficial effects with low energy diet than high energy diet, resulting in several positive responses including feed intake and milk fat content. Consequently, 0.1% NSP enzyme supplementation in lactating diet could be recommended to improve nutrient utilization of feed efficiently.

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by-products without or with xylanase supplementation. Livest. Prod. Sci. 62: 119-132.

Table 1. The formulas and chemical composition of experimental diet

Item	ME, kcal/kg	3,165		3,265	
	Enzyme, %	0	0.1	0	0.1
Ingredients, %					
Yellow corn		58.68	58.58	56.52	56.42
Wheat bran		5.00	5.00	5.00	5.00
Soybean meal		28.16	28.16	28.32	28.32
Palm kernel meal		5.00	5.00	5.00	5.00
Soybean oil		0.20	0.20	2.22	2.22
DCP		1.24	1.24	1.24	1.24
Limestone		0.92	0.92	0.90	0.90
Salt		0.50	0.50	0.50	0.50
Vit. mix[1)]		0.10	0.10	0.10	0.10
Min. mix[2)]		0.20	0.20	0.20	0.20
Multi Enzyme[3)]		-	0.10	-	0.10
Total		100.00	100.00	100.00	100.00
Chemical composition [4)]					
CP, %		18.98	18.98	18.88	18.88
C.Fat, %		3.60	3.60	5.53	5.53
C.Fiber, %		4.17	4.17	4.13	4.13
C.Ash, %		5.36	5.36	5.32	5.32
Lys, %		0.99	0.99	0.99	0.99
Ca, %		0.75	0.75	0.75	0.75
Total P, %		0.64	0.64	0.64	0.64

[1)] Provided per kg of diet : Vitamin A, 12,000 IU; Vitamin D_3, 2,400 IU; Vitamin E, 100 mg; Vitamin K_3, 3 mg; Vitamin B_1, 3 mg; Vitamin B_2, 8 mg; Niacine, 30 mg; Folic acid,4 mg; Vitamin B_{12}, 60 mg.
[2)] Provided per kg of diet : Mn, 80 mg; Zn, 30 mg; Fe, 150 mg; Cu, 5 mg; Co, 0.5 mg;
[3)] Multi enzyme : Endo-Power ® (α-galactosidase + galactomannanase + xylanase + β-glucanase; EASY BIO, Inc., Korea)
[4)] Calculated value.

Table 2. Effects of dietary energy levels and supplementation of NSP enzyme in

lactation diet on body weight and backfat thickness of lactating sows

Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Enzyme, %	0	0.1	0	0.1		Eng	Enz	ExE
No. Sows		10	10	10	10				
Body weight, kg									
24 hrs postpartum		251.2	240.8	250.2	256.5	4.35	0.409	0.189	0.346
Day 21 postpartum		244.9	237.9	247.1	254.4	4.36	0.185	0.536	0.738
Changes (0-21 d)		-6.3	-2.9	-3.1	-2.2	0.82	0.088	0.130	0.195
Back-fat thickness, kg									
24 hrs postpartum		19.5	20.3	20.9	19.3	0.28	0.699	0.441	0.027
Day 21 postpartum		18.9	20.0	20.4	19.1	0.29	0.569	0.849	0.029
Changes (0-21 d)		-0.6	-0.3	-0.5	-0.2	0.13	0.732	0.308	1.000
WEI, days		5.30	5.30	5.40	5.20	0.073	1.000	0.501	0.501
ADFI in lactation, kg		6.42	6.10	6.03	5.74	0.061	0.001	0.004	0.939

[1)] Standard error of mean.

Table 3. Effects of dietary energy levels and supplementation of NSP enzyme in

lactation diet on litter performance of lactating sows

Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Enzyme, %	0	0.1	0	0.1		Eng	Enz	ExE
No. Sows		10	10	10	10				
Litter size, no. of piglets									
Total born		11.80	11.70	11.30	12.90	0.476	0.614	0.284	0.226
Stillbirth		0.60	0.50	0.30	0.50	0.095	0.496	0.820	0.496
Mummy		0.30	0.20	0.20	0.30	0.069	1.000	1.000	0.521
Born alive		10.90	11.00	10.90	12.20	0.220	0.168	0.110	0.168
After cross-fostering		10.70	10.70	10.80	10.80	0.078	0.537	1.000	1.000
Mortality		0.40	0.20	0.30	0.20	0.071	0.739	0.322	0.739
Weaning pigs		10.30	10.50	10.50	10.60	0.095	0.425	0.425	0.789
Litter weight, kg									
At birth		16.82	17.06	17.24	15.51	2.062	0.223	0.168	0.346
After cross-fostering		16.00	15.81	16.41	15.10	3.063	0.801	0.203	0.341
21 d		54.05	54.37	55.16	56.20	5.922	0.184	0.536	0.738
Weight gain (0-21d)		38.06	38.56	38.29	40.69	5.022	0.184	0.101	0.284
Piglet weight, kg									
At birth		1.46	1.45	1.52	1.38	0.078	0.735	0.239	0.314
After cross-fostering		1.50	1.48	1.52	1.40	2.531	0.578	0.171	0.338
21 d		5.25	5.18	5.25	5.30	3.038	0.302	0.850	0.316
Weight gain (0-21d)		3.76	3.70	3.74	3.90	2.552	0.050	0.211	0.020

[1)] Standard error of mean.

Table 4. Effects of dietary energy levels and supplementation of NSP enzyme in

lactation diet on colostrum and milk composition of lactating sows

Item	ME, kcal/kg	3,165		3,265		SEM[1)]	P-value		
	Enzyme, %	0	0.1	0	0.1		Eng	Enz	ExE
Fat, %									
Colostrum		6.72	6.72	6.72	6.72	0.242	-	-	-
Milk at 21 d		7.44	7.89	7.53	7.94	0.131	0.814	0.141	0.956
Protein, %									
Colostrum		10.53	10.53	10.53	10.53	0.559	-	-	-
Milk at 21 d		5.46	5.38	5.62	5.45	0.088	0.626	0.657	0.208
Lactose, %									
Colostrum		3.73	3.73	3.73	3.73	0.149	-	-	-
Milk at 21 d		5.30	5.42	5.66	5.84	0.110	0.091	0.510	0.900
Total solid, %									
Colostrum		22.66	22.66	22.66	22.66	0.672	-	-	-
Milk at 21 d		19.94	20.28	20.25	20.11	0.230	0.898	0.861	0.652
Solid not fat, %									
Colostrum		13.07	13.07	13.07	13.07	0.264	-	-	-
Milk at 21 d		11.20	11.48	11.28	11.42	0.099	0.982	0.279	0.731

[1)] Standard error of mean.

Chapter V: Effects of Energy Modulation and NSP Enzyme Supplementation on Nutrient Digestibility of Gestating Sows

ABSTRACT: The experiment was conducted to investigate the effect of dietary energy levels and enzyme supplementation on nutrient digestibility of gestating sows. Four multiparous sows (Yorkshire × Landrace) were allotted in a repeated 4 × 4 Latin-square design with a 2 x 2 factorial arrangements. Four treatments were arranged with 2 main factors of energy levels (3,165 or 3,265 kcal of ME/kg) or enzyme supplementation (0 or 0.1% of NSP enzyme; α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea). After 5 days of adaptation, the excreta were collected for 4 days to analyze the digestibilities of dry matter, protein, ash and fat. There was no significant difference in the results of body weight and backfat thickness during the whole collection period, but numerically improved changes of backfat thickness were detected when sows were fed diet containing 3,165 kcal of ME/kg with 0.10% NSP enzyme compared with those fed low energy diet without NSP enzyme. When sows were fed high energy diets, improved digestibilities of ash and fat were observed relative to those fed low energy diets (energy response, $P<0.01$), and the interaction effect between dietary energy level and supplementation level of NSP enzyme was observed in the digestibilities of dry matter and ash (interaction effect, $P=0.07$ for dry matter and $P<0.05$ for ash), resulted in higher effects of supplementing NSP enzyme in sows fed low energy diet. This study demonstrated that supplementing NSP enzyme contributed a positive effect on digestibilities of dry matter, crude fiber and ash when sows were fed low energy diet (3,165 ME kcal/kg).

Key words: Sow, Energy Level, NSP Enzyme, Nutrient Digestibility

INTRODUCTION

The livestock industries have some challenges how to improve animal digestibility and maximize body weight gain. So, these challenges were pursued for competitive ability of farms and livestock industries. Practically, many researchers have efforts to develop some enzymes and probiotics for digestibility improvement.

In general, NSP (non-starch polysaccharides) is perceived as anti-nutritional factor to lower the feedstuff's value. As NSP has different composition and categories by ingredients. It was sorted to α-galactoside, β-mannan (galactomannan), pentosans (arabinoxylan and xylan), and β-glucan that decreased the digestibility, feed efficiency, body weight gain and increased incidence of diarrhea. Therefore, improved availability of NSP by NSP enzymes supplemented in diet has positive effects on feed efficiency, energy availability and economical benefits because NSP induces the loss of metabolizable energy. To improve the NSP availability, enzymes are necessary factor for improving the nutrient digestibility and feed efficiency and large biological molecules responsible for the thousands of chemical interconversion. They are highly selective catalysts, greatly accelerating both the rate and specificity of metabolic reactions. Enzyme activity can be affected by temperature, pressure, chemical environment (e.g., pH). Subsequently, it is desirable to choose the enzymes that have specificities to several substrates and high activity within environmental conditions.

In weaning pigs (21 days), the level of some enzymes secretion had still lowed at 10 d before weaning (Miller et al., 1986). Texas Tech. University demonstrated that weaning pigs fed diets containing enzyme complex (Endo-Power®: α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea) showed higher body weight gain (7%) and feed efficiency (9%) compared to control treatment. The University of Illinois presented that weaning pigs fed diet containing 0.1% enzyme showed increased energy digestibility (7%)

and amino acid digestibility (3%), and the University of Texas Tech. reported that weaning pigs fed diet containing 0.1% enzyme had improved digestibility of starchyose and raffinose. In growing-finishing pigs, Texas Tech. University reported that digestibility of amino acid and energy was improved from 2 to 5%, and Kim et al. (2006) also indicated that the feed efficiency of finishing pigs was improved despite of 3% decrease in dietary energy levels when enzyme was added to diets. Although many experiments were conducted for weaning pigs and growing-finishing pigs, little information was available for sows.

Therefore, this study was conducted to investigate the effects of dietary energy levels and enzyme supplementation in gestation diet on the nutrient digestibility of gestating sows.

MATERIALS AND METHODS

Experimental Design and Diet

Four multiparous sows (BW=260.5±19.3, Yorkshire × Landrace) were randomly assigned in a repeated 4 × 4 Latin-square design balanced for carryover effects. To evaluate factors effects, four dietary treatments were designed with a 2 × 2 factorial arrangement. The first factor is energy levels in diet (3,165 or 3,265 kcal of ME/kg), and second factor included supplementation of 0.1% NSP enzyme complex (α-galactosidase + galactomannanase+ xylanase + β-glucanase; EASY BIO, Inc., Korea). All experimental diets were formulated to meet or exceed nutrient requirement of NRC (1998), and contained 12.90% crude protein, 0.61% lysine, 0.22% methionine, 0.75% calcium, and 0.67% total phosphorous respectively. The experimental diet formula and chemical composition were given in Table 1.

Animal Housing, Data Collection and Digestibility Trial

At 28 d after pregnancy diagnosis, the 4 gestating sows were housed in an individual metabolic crate (2.5×1.8 m^2, height 0.50 m). Each treatment diet was provided 2.4 kg/day twice daily with water to gestating sows. Chromic oxide was mixed at 0.5% of the diet as indigestible markers. After a 5-day adaptation period, total amount of feed consumed and excreta were recorded daily for 4 days. Collected excreta from each sow were pooled, sealed in plastic bags and kept frozen at -20 °C. After air-forced oven dried at 60 °C for 72 h, those samples were ground by a Wiley mill to pass 1 mm screen for chemical analysis. The methods of AOAC (1995) were used for chemical analysis of experimental diets and excreta. The body weight and backfat thickness (BFT) were recorded to measure their changes during the digestibility trial. Ultra-sound (Lean-meter, Renco Corp., Minneapolis, USA) was used for measuring BFT at P_2 position (mean value from both side of the last rib and 65 mm away from the backbone).

Statistical Analysis

All of collected data were carried out by least squares mean comparisons and evaluated using PDIFF option with the General Linear Model (GLM) procedure of SAS (SAS Institute, 2004). Individual sows and their litters were used as an experimental unit and analyzed as a 2 x 2 factorial arrangement, and differences were declared significant at $P<0.05$ and highly significant at $P<0.01$.

RESULTS

Effects of dietary energy levels and NSP enzyme supplementation on body weight and backfat thickness of gestating sows during collection period were shown in Table 2. During the whole collection period, there was no significant difference on the results of body weight and backfat thickness, but numerically improved changes of backfat thickness were detected in sows fed diet containing

3,165 kcal of ME/kg and 0.1% NSP enzyme (-0.25mm) relative to those fed low energy diet without NSP enzyme (-0.75mm). In the results of digestibility trial, digestibilities of all parameter tended to be improved when high energy diets were fed to gestating sows (energy response, $P<0.1$). In case of crude fiber, significant increase of digestibility was affected by NSP enzyme supplementation regardless of energy level ($P<0.01$), also NSP enzyme showed improving tendency in crude protein digestion ($P=0.09$). And the interaction effect between dietary energy levels and supplementation levels of NSP enzyme was observed in the digestibilities of ash (interaction effect, $P=0.08$).

DISCUSSION

During the whole collection period, body weight and backfat thickness of gestating sows were not changed significantly because the digestibility trial was conducted in early gestation. Improved feed efficiency and fat digestibility by addition of fat sources such as soybean oil and tallow have been reported frequently in previous findings (Crampton and Ness, 1954; Frobish et al., 1970; Allee et al., 1971; Atteh and Leeson, 1983; Lawrence and Maxwell, 1983), and the ability of pigs to digest fat was associated with fat source and improved as age increased (Cera et al., 1990). The results of the present study are consistent with previous findings, wherein the sows fed high energy diets showed higher fat digestibility than those of low energy treatment (energy response, $P<0.01$). That may be due to the energy source using soy oil for elevating energy level. Little information is available about the effects of dietary energy level on ash digestibility of gestating sows, and the ash digestibility of gestating sows was increased as dietary level elevated in this study. Although mechanism for increasing ash digestibility by increasing dietary energy level is unknown yet, this explanation may be due to digestion efficiency from improved fat digestibility. Moreover, high energy diet

contained slight more dehulled soybean meal compared to low energy diet for contributing protein requirement in the formula, it could be a positive factor which is related improvement of several nutrient digestibilities.

Even though addition of NSP enzyme to gestation diet had no effects on the digestibilities of dry matter, fat and ash, the effect of supplementing NSP enzyme to gestation diet on ash digestibilities of gestating sows was increased when sows were fed low energy diets relative to those of high energy treatments (interaction effect, P=0.08). That means addition of NSP enzyme had a positive effect on ash digestibilities in sows fed low energy diet. These results of low energy treatments are consistent with results of Omogbenigun et al. (2004), Fang (2007), Li et al. (1996). Also, Baucells et al. (2000) and Moeser and Kempen (2002) suggested the positive effects of NSP enzyme on nutrient digestibility of pigs, which is indicating that digestibilities of dry matter and energy were increased respectively (2% for DM digestibility and 3% for energy digestibility) by the addition of NSP enzyme in corn-SBM based diet. However it was contrasted to the results of Petty et al. (2002) and Olukosi et al. (2007) that demonstrated no detectable effects of NSP enzyme addition on nutrient digestibility. Ao et al. (2010) demonstrated that the apparent digestibility of dry matter was improved when NSP enzyme was added to diet in growing pigs. Many experiments showed inconsistent results caused by different composition of diet and age of animals. Also, Kim (2003) represented that the reasons of these inconsistent results were differences derived from ingredient of enzyme, storage, feeding process and healthy of animal. Several researchers reported viscosity in the intestine was associated with enzymes, viscosity was decreased by enzyme treatment (Sudendey and Kamphues, 1995). And low viscosity resulted in higher digesta passage rate in the intestine. Viscous non starch polysaccharides without NSP enzyme may also physically coat starch granules, further reducing the rate of digestion (Ellis et al., 1996)

CONCLUSION

Addition of NSP enzyme to gestation diet had no significant effects on body weight and backfat thickness of sows in early gestation, and the sows fed high energy diets showed higher fat digestibility relative to those fed low energy diets. The NSP enzyme addition improved the digestibility of fiber significantly, also showed tendency to increase protein digestibility. Interaction effect was observed on the digestibility of ash, resulted in improved response of enzyme addition of sows fed low energy diets.

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Table 1. The formulas and chemical composition of experimental diet

Item	ME, kcal/kg	3,165		3,265	
	Enzyme, %	0	0.1	0	0.1
Ingredients, %					
Yellow corn		61.81	61.71	58.83	58.73
Wheat bran		7.46	7.46	8.06	8.06
Rice bran		5.00	5.00	5.00	5.00
Beet pulp		4.00	4.00	4.00	4.00
Soybean meal		12.38	12.38	12.62	12.62
Palm kernel meal		5.00	5.00	5.00	5.00
Soy oil		1.62	1.62	3.76	3.76
Choline-Cl		0.09	0.09	0.09	0.09
TCP		1.34	1.34	1.34	1.34
Limestone		0.60	0.60	0.60	0.60
Salt		0.50	0.50	0.50	0.50
Vit. mix[1)]		0.10	0.10	0.10	0.10
Min. mix[2)]		0.10	0.10	0.10	0.10
Multi Enzyme[3)]		-	0.10	-	0.10
Total		100.00	100.00	100.00	100.00
Chemical composition [4)]					
CP, %		13.00	13.00	13.00	13.00
C.Fat, %		5.33	5.33	7.38	7.38
C.Fiber		4.50	4.50	4.50	4.50
C.Ash, %		5.21	5.21	5.22	5.22
Lys, %		0.62	0.62	0.62	0.62
Ca, %		0.75	0.75	0.75	0.75
Total P, %		0.67	0.67	0.67	0.67

[1)] Provided per kg of diet : Vitamin A, 12,000 IU; Vitamin D_3, 2,400 IU; Vitamin E, 100 mg; Vitamin K_3, 3 mg; Vitamin B_1, 3 mg; Vitamin B_2, 8 mg; Niacine, 30 mg; Folic acid,4 mg; Vitamin B_{12}, 60 mg.
[2)] Provided per kg of diet : Mn, 80 mg; Zn, 30 mg; Fe, 150 mg; Cu, 5 mg; Co, 0.5 mg;
[3)] Multi enzyme : Endo-Power ® (α-galactosidase + galactomannanase + xylanase + β-glucanase; EASY BIO, Inc., Korea)
[4)] Calculated value.

Table 2. Effect of dietary energy levels and NSP enzyme supplementation on body weight and backfat thickness of gestating sows during collection period [1)]

Item	ME, kcal/kg	3,165		3,265			P-values		
	Enzyme, %	0	0.1	0	0.1	SEM[2)]	Eng	Enz	ExE
Body weight, kg									
Initial		271.5	273.6	273.1	272.3	8.04	0.991	0.957	0.897
Final		275.2	278.7	277.2	276.4	7.75	0.986	0.906	0.845
Change		3.7	5.1	4.1	4.1	1.17	0.854	0.687	0.687
Back-fat thickness, mm									
Initial		19.38	18.88	18.88	19.25	0.67	0.948	0.948	0.655
Final		18.63	18.63	18.88	19.50	0.55	0.490	0.699	0.699
Change		-0.75	-0.25	0.00	0.25	0.29	0.157	0.383	0.768

[1)] N=16 (4 sows per each treatment)

[2)] Standard error of mean

Table 3. Effect of dietary energy levels and NSP enzyme supplementation on the nutrient digestibility of gestating sows[1)]

Item	ME, kcal/kg	3,165		3,265			P-values		
	Enzyme, %	0	0.1	0	0.1	SEM[2)]	Eng	Enz	ExE
Dry matter, %		82.04	83.77	84.13	84.29	0.52	0.10	0.23	0.31
CP, %		77.04	79.76	80.13	81.35	0.74	0.05	0.09	0.49
C.Fat, %		88.96	85.94	91.80	90.34	1.21	0.06	0.22	0.66
C.Fiber, %		57.27	65.86	62.25	67.01	1.02	0.06	<0.01	0.21
C.Ash, %		29.10	38.51	40.33	38.33	2.12	0.09	0.24	0.08

[1)] N=16 (4 sows per each treatment)

[2)] Standard error of mean

Chapter VI. Overall Conclusion

Recently, the findings to evaluate the effects of NSP enzyme has been concentrated on the piglets because the digestive system of piglets is incomplete compared with those of adult pigs. However, little information is available about the supplementation effects of NSP enzyme in gestating and lactating sows. Therefore, three experiments were conducted to evaluate 1) the effect of energy modulation and NSP enzyme (Endo-Power®) supplementation for gestation diet on physiological responses and reproductive performance of sows, 2) the effect of energy modulation and NSP enzyme (Endo-Power®) supplementation for lactation diet on physiological responses and reproductive performance of lactating sows, and 3) the effect of energy modulation and NSP enzyme (Endo-Power®) supplementation on nutrient digestibility of gestating sows.

Supplementing NSP enzyme to gestation diet had no effects on the body weight, backfat thickness, and ADFI in lactation, and the sows fed diet containing NSP enzyme showed numerically increased weight gain from 0 to 110 d of gestation, and decreased losses of body weight and voluntary feed intake in lactation (0-21 d). Then, this numerical difference was increased when the sows fed diet containing 3,165 kcal of ME/kg relative to those fed diet containing 3,265 kcal of ME/kg. The sows fed high energy (energy response, $P<0.01$) and enzyme diets (enzyme response, $P<0.03$) showed increased average weight of piglets at birth, and those fed diet containing 3,265 ME kcal/kg and 0.1% NSP enzyme showed numerical decrease of litter size without significant difference.

The supplementation of NSP enzyme to lactation diet is not associated with the body weight, backfat thickness, WEI, milk composition of lactating sows and growth performance of nursery piglets. But sows fed enzyme diets showed

lower ADFI relative to those without enzyme, suggesting enzyme supplementation could compensate nutrients utilization during lactation period.

Sows fed high energy diets showed improved fat and protein digestibilities compared to those fed low energy diets. On the other hand, NSP enzyme addition increased digestibilities of protein and fiber. Their interaction was observed in digestibility of ash, resulted in improved response of enzyme addition of sows fed low energy diets.

Consequently, high energy diet increased body weight gain during gestation and decreased body weight loss during lactation period. Enzyme supplementation compensated body condition score in low energy diet during gestation period, increased litter weight gain during lactation period. Physiological and reproductive performance represented inconsistent responses by energy level and enzyme. However, supplementation of NSP enzyme (Endo-Power®) or high energy to the diet in gestating sows increased nutrient digestibilities. These results imply NSP enzyme could be supplemented with reduction of energy for the sow diet formulation to save feed cost effectively.

Chapter VII. Summary in Korean

본 실험은 임신돈 사료 내 에너지 함량 및 효소제 첨가유무가 모돈의 생리적 반응, 번식성적, 유성분, 영양소소화율 및 포유자돈의 성장성적에 미치는 영향과 포유돈 사료 내 에너지 함량 및 효소제 첨가유무가 포유돈의 생리적 반응, 사료섭취량, 유성분 및 포유자돈의 성장능력에 미치는 영향을 평가하기 위해 수행되었다.

Experiment I. Effects of Energy Modulation and NSP Enzyme Supplementation in Gestation Diet on Physiological Responses and Reproductive Performance of Sows

본 실험은 임신기 사료 내 에너지 함량 및 복합 NSP효소의 첨가가 임신기 및 포유기 모돈에 미치는 영향을 규명하고 복합효소제의 에너지 보상효과를 검증하기 위해 수행되었다. 실험은 사료의 에너지 함량 (3,165 또는 3,265 kcal of ME /kg)과 복합효소제의 첨가 유무에 따라 2 × 2 요인실험으로 설계되었으며, 평균체중 216.9 ± 51.6 kg의 F1 교잡종 (Yorkshire × Landrace) 경산 모돈 48두를 공시하여 체중과 등지방 두께에 따라 4개 처리에 완전임의 배치법 (CRD: completely randomized design)으로 배치하였다. 실험결과, 임신기 모돈의 체중 및 등지방에서 처리구에 따른 유의적인 차이가 나타나지 않았으며, 사료 내 에너지 함량이 증가될 경우 임신기 동안의 증체량이 증가하는 경향이 발견되었다 (energy response, P=0.09). 이외에 포유기 모돈의 체중, 등지방 및 사료섭취량에서도 처리구에 따른 유의차가 나타나지 않았지만, 효소제를 첨가함에 따라 포유 모돈의 체중 및 등지방

감소량이 수치적으로 줄어드는 경향을 나타냈으며, 이러한 경향은 저에너지 사료를 모돈에게 급여하였을 경우 더욱 두드러졌다. 에너지 수준이 3,265 kcal of ME/kg인 사료를 급여하거나 (energy response, $P<0.01$) 효소제를 공급해주었을 경우 (enzyme response, $P<0.05$) 생시 자돈의 평균 체중이 유의적으로 증가되는 것으로 나타났다. 임신기 사료 내 에너지 함량 및 복합 NSP효소의 첨가가 모돈의 혈액성상에 미치는 영향을 분석한 결과, 효소제를 공급해주었을 때 임신 70일령의 혈중 인슐린 함량이 증가되는 것으로 나타났으며 (enzyme response, $P<0.05$), 고 에너지 사료를 급여하였을 경우 임신 110일령의 혈중 포도당 함량이 감소되는 것으로 나타났다 (energy response, $P<0.05$). 모유 성분분석 결과, 분만 후 7일령의 유당함량이 임신기 사료 내에 효소제를 첨가하였을 경우 유의적으로 증가되는 것으로 나타났으며 (enzyme response, $P<0.05$), 분만 후 14일령의 무지유고형분 함량에서 저에너지 사료를 공급받았을 경우 효소제의 효과가 두드러지게 나타나는 경향이 발견되었다 (interaction effect, $P=0.05$). 이러한 경향은 임신 90일령 및 110일의 체평점 지수에서도 동일하게 나타났다 ($P<0.10$). 결론적으로 사료 내 에너지 함량과 효소제는 모돈의 번식성적 및 포유자돈의 생시체중 등에 밀접한 관련이 있는 것으로 나타났으며, 사료 내 에너지 함량이 감소될 경우 효소제의 첨가효과가 더욱 향상되는 것으로 나타났다.

Experiment II. Effects of Energy Modulation and NSP Enzyme Supplementation in Lactation Diet on Physiological Responses and Reproductive Performance of Lactating Sows

본 연구는 포유 모돈 사료 내 NSP 효소제의 급여가 모돈의 번식 성적, 사료섭취량 및 포유 성적에 미치는 영향을 규명하기 위해서 수행되었다. 실험은 사료 내 에너지 함량 (3,165 또는 3,265 kcal of ME/kg)과 복합 효소제의 첨가 유무에 따라 2 × 2 요인실험으로 설계되었으며, 평균체중 249.7 ± 8.86 kg의 F1 교잡종 (Yorkshire × Landrace) 경산 모돈 40두를 공시하여 분만 직후에 체중과 등지방 두께에 따라 4개 처리에 완전임의 배치법 (CRD: completely randomized design)으로 배치하였다. 실험 결과, 포유 기간 동안의 사료 내 에너지 수준이 높을 경우(P=0.09)와 효소제를 사용하는 경우(P=0.13) 모돈의 체중 감소가 덜 발생하는 경향이 발견되어 체손실은 개선되었으며, 포유 21일령의 등지방 두께에서 저에너지 사료를 공급받았을 경우 효소제의 효과가 두드러지게 나타나, 사료 내 에너지 함량 및 효소제 첨가 유무에 따른 상호효과가 발견되었다 (interaction effect, P<0.05). 반면 포유기 사료 내 에너지 함량을 높여주거나 (energy response, P<0.01) 효소제를 급여할 경우 (enzyme response, P<0.01) 포유기 모돈의 사료섭취량은 감소되는 것으로 나타났으며, 돈유 성분 및 자돈의 성장성적에는 처리구에 따른 변화가 없는 것으로 나타났다. 결론적으로, 포유 모돈 사료 내에 효소제를 첨가할 경우 모돈의 체손실은 감소되고, 사료이용성은 개선시킬 수 있는 것으로 나타났다.

Experiment III. Effects of Energy Modulation and NSP Enzyme Supplementation on Nutrient Digestibility of Gestating Sows

본 연구는 임신돈 사료 내 NSP 효소제의 첨가가 모돈의 영양소 소화율에 미치는 영향을 평가하기 위해 수행되었다. 실험은 사료의 에너지 함량 (3,165 또는 3,265 kcal of ME/kg)과 복합효소제의 첨가 유무에 따라 2 × 2 요인실험으로 설계되었으며, 평균체중 260.5 kg의 F1 교잡종 (Yorkshire × Landrace) 임신 모돈 4두를 공시하여 체중 및 등지방 두께에 따라 4 x 4 라틴 방각법 (Latin square design)으로 배치하였다. 실험 전기간 동안 임신 모돈의 체중 및 등지방에서 처리구에 따른 유의적인 차이가 나타나지는 않았지만, 에너지 함량이 3,165 kcal of ME/kg인 사료를 섭취한 모돈의 경우 효소제를 공급받았을 때 등지방 감소량이 수치적으로 줄어들었다. 소화율 실험 결과, 사료 내 에너지 함량이 증가될 경우 조단백질의 소화율은 유의적으로 개선되었으며($P=0.05$), 전체적인 영양소 소화율이 개선되는 경향이 나타났다(energy response, $P<0.1$). 특히 NSP 효소제는 섬유소 소화율에 있어 고도의 유의적 개선 효과가 나타났으며(enzyme response, $P<0.01$), 단백질의 소화에도 도움을 주는 경향이 확인되었다($P=0.09$). 반면, 회분 소화율 (interaction effect, $P=0.08$)에서 저에너지 사료를 공급받았을 경우 효소제의 효과가 부각되어 사료 내 에너지 함량 및 효소제 첨가에 따른 상호효과가 발견되었다. 결론적으로, 사료 내 에너지 함량이 3,165 kcal of ME/kg 수준일 경우 NSP 효소제를 첨가할 시 단백질 및 섬유소의 소화율을 개선시킬 수 있을 것으로 나타났다.

Acknowledgement

I would like to express my gratefully and sincerely respect to Dr. Yoo Yong Kim for his invaluable guidance and unceasing devotion throughout doctoral course. He always made great efforts to apply scientific contribution and develop competitive swine industry. Moreover I wish to appreciate his paramount mentorship for students.

These studies were supported and funded by the EASY BIO, Inc. in Korea. I sincerely appreciate to Won Chul Ji and Ji Beom Kim for their full supports and thoughtful considerations.

I also wish to express deep appreciation to Drs. Myung Gi Baik and Chung Soo Chung for their deep understanding and encouragement. A special appreciation should be to Dr. Kyung Hoon Cho for thoughtful guidance to industrial application. Heartily, I owe special thanks to Dr. Young Hyun for many helps and advices to these Ph. D. study, he actively participated from beginning to end.

I am very pleased by my research group members and fellow students at the Laboratory of Animal Nutrition and Biochemistry. I will always remember their helps and the memories with them.

Also, I sincerely thank to all of family, parents (Yun, Jeong Un and Cheong, Gyu Jin) and parents in law (Kim, Sam Deuk and Lee, Gap Suk) for their trust.

I really wish to give the greatest gratitude to my beloved sweetheart (Kim, Ji Eun) and my cute angels (Hakyeong and Sowon) for their endless devotion, encouragement and love.

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February, 2014