A Thesis

For the Degree of Master of Science

Effect of β-mannanase (Hemicell-HT®) Supplementation, as an Alternative toward Antibiotics, on Growth Performance and Intestinal Integrity in Weaning Pigs

항생제 대체제로서 β-mannanase의 첨가가 이유자돈의 성장능력 및 소장의 건강성에 미치는 영향

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이 논문을 농학석사 학위논문으로 제출함

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Summary

Dietary antibiotics have been used for over 50 years to treat or prevent disease in swine industry. However, because of antibiotic-resistant pathogens and antibiotic residue problems, most of swine producing countries (including Korea) officially prohibited antibiotic application to animal feed. For that reason, lots of feed additives including dietary enzymes were produced as an alternatives toward antibiotics. As a part of this, a research was conducted to evaluate the effect of Hemicell-HT® supplementation on growth performance and intetinal integrity in weaning pigs as an alternative feed additive toward dietary antibiotics. A total of 96 weaning pigs (28 ± 3 old and 6.96 ± 0.70 kg of BW) were allotted into 4 treatments with 6 replicates of 4 piglets per pen in a randomized completely block (RCB) design. Treatments were: 1) NC (negative control) (basal diet), 2) PC (basal diet + 0.1 % antibiotics), 3) A (basal diet + 0.04 % Hemicell-HT®), 4) B (basal diet + 0.06 % Hemicell-HT®). Two phase feeding programs (Phase I for 0 - 2 week and Phase II for 3 - 5 week) were used in this experiment. In feeding trial, PC and B treatments showed significantly higher growth performance than any another treatments. There were no significant difference in the glucose, lactate, triglycerides, (acute phase protein) APP level. In nutrient digestibility, pigs fed 0.06% of Hemicell-HT® showed significantly higher crude fat digestibility. The ratio of villi height and crypt depth in jejunum and ileum was higher when pigs were fed 0.06% Hemicell-HT® treatment diet. In moisture contents of feces, pigs fed diet with antibiotics had significantly lower moisture of feces ($P < 0.05$) but, 0.04% Hemicell-HT® treatment showed statistically similar moisture contents compared to positive control in all phases. Based

upon this experiment, Hemicell-HT® can be supplemented in weaning pig's diet without growth check or detrimental effect although growth performance was lowered slightly compared to antibiotics treatment.

Key words : Hemicell-HT®, β-mannanase, weaning pig, Growth performance, Nutritient digestibility, Intestinal integrity

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List of Abbreviation

AA	Amino acids
AOAC	Association of official analytical chemists
ADG	Average daily gain
ADFI	Average daily feed intake
AGP	Animal growth promoters
ATP	Adenosine triphoshpate
BW	Body weight
CP	Crude protein
DM	Dry matter
EU	European union
FMD	Food and mouth disease
FTA	Free trade agreement
GIT	Gastrointestinal tract
GLM	General linear model
IGF	Insulin-like growth factor
LSD	Least significantly difference
ME	Metabolizable energy
MOS	Mannan oligosaccharaides
NDF	Neutral detergent fiber
NRC	National Research Council

PUFA	Polyunsaturated fatty acid
NSP	Non-starch polysaccharide
PWC	Post weaning colibacillosis
PWS	Post weaning syndrome
RCB	Randomized complete block
SAS	Statistical Analysis System
SBM	Soybean meal

I. Introduction

These days, Korean swine industries have numerous troubling factors such as EU (European Union) - Korea FTA (Free trade agreement), the issue of dump animal excrement into the sea, FMD (foot and mouth disease) and PMWS (post-weaning multi-systemic wasting syndrome) have been negatively influenced to the swine breeders. In order to overcome these circumstances, researchers are keep studying and focusing on the increasing animal growth performance and decreasing mortality rate of weaning pigs. For such reasons, antibiotics or antibiotic-like growth promoters, are commonly used in weaning and growing pig's diets. In recent years, however, pig breeders and veterinarians across the globe are feeling the pressure to reduce antibiotic medicines to treat sick livestock after a recent spread of antibiotic-resistant disease in humans. Those concerns that development of resistant pathogen strains in animal and antibiotic residues in animal products has led to pressure to make plans and new tools. finally, supplementation of antibiotics in animal feed as an animal growth promoter (AGP) has been completely banned since 2011 in European Union. The Republic of Korea also prohibited the use of antibiotics in swine diets since July, 2011. In order to solve these circumstances, many of the swine researchers and nutritionists have been searching for alternative substances which can be used in stead of antibiotics in animal feed.

On the surface, adding dietary enzymes to swine diets has sharply increased, and interest in use of various types of dietary enzymes in swine diets to improve nutrient digestibility has

tremendously increased in recent years. Enzymes are used in animal diet to stimulate nutrient digestion and eliminate or minimize the negative effects on specific components of feed ingredients on nutrient digestion.

Hemicelluloses, classified as nonstarch polysaccharides, are known to present in the cell wall structure of ungerminated leguminous seeds. Many of these seeds, including soybeans, are used in weaning pigs diets as protein sources and can contain up to 22.7% non-starch polysaccharides (NSP) content on a DM (dry matter) basis (Chesson, 1987). These NSPs have been shown to diminish growth performance and inhibit nutrient absorption in poultry (Vorha and Kratzer, 1964; Verma and McNab, 1982) and swine (Blackburn and Johnson, 1981; Rainbird et al., 1984; Edwards et al., 1988). The presence of β-mannan from guar gums in the diet has also been shown to reduce gastric emptying and inhibit the secretion of insulin, glucose-dependent insulinotrophic peptide (GLP), and insulin-like growth factor (IGF-I) (Nunes and Malmlof, 1992).

β-mannanase is an endohydrolyase enzyme that is a fermentation product of *Bacillus lentus*, which degrades β-mannans. The enzyme cleaves randomly within the 1, 4-β-mannan main chain of galactomannan, galactoglucomannan, and mannan (McCleary, 1988).

In several recent studies, β-mannanase addition enhance the feeding nutritional values of soybean-meal (SBM) (Coon et al., 1990). Significant effects of β-mannanase on the performance of laying hens (Jackson et al., 1999), swine (Hahn et al., 1995; O'Quinn et al., 2002; Petty et al., 2002), and turkeys (Odetallah et al., 2002) have been

reported. There exists evidence that β-mannanase can improve body weight uniformity in several monogastric species (Anderson et al., 2001). The hypothesis of this study is that the corn-SBM diets including β-mannanase fed to weaning pigs shows similar effect compare with the antibiotics supplementation.

II. Literature Review

1. Introduction

Antibiotics have been used for more than 50 years to enhance growth performance and to prevent numerous diseases in livestock feeding environments. The AGP have commonly been used as growth stimulants and to treat gastrointestinal infections sub-therapeutically (Verstegen and Williams, 2002). Experience has told us that because the use of antibiotic growth promoters is thought to cause antimicrobial resistance, it is not a sustainable option in the long run. It has resulted in the ban of AGP in animal nutrition in the EU and Korea as well. In the event that use of antibiotics in commercial swine feeding operations are restricted, many animal scientists have been investigating natural alternatives to conventional chemotherapeutical agents.

This review firstly focuses on antibiotics itself, a brief mention on the characteristics of its usage, as treatment, control, history and recent trend of antibiotic use in the animal industry. Then, it moves on to possible alternatives to antibiotics and feed enzymes. It may enhance growth performance and nutrient digestibility by degrading mannan in NSPs which act as anti-nutritional factors in swine feed ingredients. Lastly, the review will introduce mode of action of enzyme and effect in weaning pigs growth performance.

2. Growth of the weaning pigs

The weaning age of pigs has been reduced from about 8 weeks of age in the 1950s and 1960s down to a current average weaning age

of 21-28 days of age that is practiced in many pig-producing countries. In some instances, even earlier weaning ages (< 21 days) are adopted using different methods. The reduction in weaning age has occurred largely because of the productivity increases, both in the growing and breeding herds, which were achievable. However, the inevitable shift to earlier weaning ages presented many problems concerning the nutrition, housing, health, behavioral and environmental requirements of the young pig, as well as having consequences for the fertility of the sow. These are especially pertinent in systems where pigs are weaned at less than 21 days of age, such as segregated early weaning (SEW) practices. Much research, combined with field experience, has minimized the stressors encountered at weaning so that good levels of production can be achieved after weaning.

2.1 The ecology of gastrointestinal tract (GIT) at weaning

The microbial population of young pigs develops during the first 48 hours of life via the ingestion of maternal feces (Makie et al., 1999) or by the contact with the sow skin and teats that are usually contaminated (Arboukle, 1968). During the suckling period, the dominant groups in the upper part of the gastrointestinal tract are lactobacilli and streptococci (Jensen, 1998). At birth, the stomach of piglets is sterile (Sinkovics and Juhasz, 1974), but after few hours viable cells of lactobacilli, streptococci, coliforms and clostridia, can be isolated from the gastric content. During the following 5-6 days, the number of lactobacilli and streptococci is around 10^5-10^7 cells per gram of content (Jensen, 1998; Pluske, 2002) and it will be stable for all the suckling period. In the proximal small intestine there is a microflora similar to that of stomach, being therefore lactobacilli and streptococci

the dominant groups (Jensen, 1998). In contrast, the distal small intestine, contains an higher number of bacteria (10^8-10^9 cells per gram of digesta). Although determining the exact composition of the large intestine microflora is very hard, because of the high number of unculturable cells, King an Kelly (2001) found 10^{10}-10^{11} cells per gram of content in the lumen of colon, with more than 400 different colturable bacterial species. Numerous studies conducted on the characterization of large intestine microflora demonstrated that the maior groups are *Streptococcus, Lactobacillus, Prevotella, Selenomona, Mitsokuella, Megasphera, Clostridia, Eubacteria, Bacteroides, Fusobacteria, Acidodaminicocci,* and *Enterobacteria* (Salanitro et a., 1977; Allison et al., 1979; Russel, 1979; Robinson et al., 1981; Moore et al., 1987; Jensen, 2001; and Pluske et al., 2002). Although detailed studies on the microflora in the hindgut have been conducted, there is evidence of a great diversity between animals and between different resulting studies since the variability in number or type of cells depends on the nutrients availability in the gut, being nutrients limiting factors which support the growth of one bacterial strain rather than another one (Gaskins, 2001).

At weaning there are many changes which interfere with the normal microbial populations. Jensen (1998) investigated the effect of weaning on microbial counts, pH, and dry matter of digesta. In that study, it was observed that during the first 2 to 4 days after weaning pH and dry matter content of fecal samples and lactobacilli counts decreased, whereas, concurrently, coliforms counts increased. This tends to make the pig susceptible to diarrhea scouring and poor growth performance. In the same study, piglets were sacrificed at weaning (28

days), 6 days and 20 days after weaning, and samples from different intestinal tracts were collected to measure pH, dry matter, and microbial determination and activity through adenosine triphosphate (ATP) concentration. The results demonstrated that the microflora of piglets after 6 days from weaning was different from the other groups, since there was a higher number of coliforms cells and a lower number of lactobacilli in each intestinal segment. Animals sacrificed 20 days after weaning presented a significantly higher microbial activity in the large intestine, as demonstrated by lower pH and higher ATP concentration. with these results conducted, Jensen to concluded that the microbiota of piglets is instable until 3 weeks after weaning, when the large intestine fermentative capacity is fully developed.

2.2 Health implication of microflora and impact of antibiotics on the GIT

The major health implications of the gut flora vary widely ranging from the production of toxic, carcinogenic or mutagenic metabolites from substances derived from the diet or produced endogenously, to the detoxification of dietary toxicants, to immunostimulation (Link-Amster *et al*., 1994; Marteau *et al*., 1997), to intestinal permeability (Isolauri *et al*., 1993), and to confer colonization resistance towards pathogens and a consequent prevention of diarrhoea (Raza *et al*., 1995).

Intestinal fermentations prevalently occur in the hind gut (Decuypere and Van der Heyde, 1972) where decarboxylation of several amino acids by bacteria can produce monoamines and polyamines (Dierick *et al*., 1986). They can exert toxic effects in different species

(Lean *et al*., 1989; Cole *et al*., 1995). Furthermore, the intestinal microflora is also deeply involved in the production of ammonia. Ammonia is produced both by endogenous and bacterial enzymes within the alimentary tract. Bacterial enzymes appear to produce 75% of the alimentary tract ammonia with urea hydrolysis being the major contributor in mammals residing in conventional nongerm-free environments (Visek, 1984). Energy is the fermentation limiting factor in the hind gut (Orskov et al., 1970). As energy sources (starch and fermentable carbohydrates) are depleted, the fermentation becomes more and more proteolytic. This results in ammonia and amines production (Russell et al., 1983); ammonia destroys cells, alters nucleic acid synthesis, increases intestinal mucosal cell mass, increases virus infections, favors growth of cancerous cells over noncancerous cells in tissue culture (Visek, 1978) and reduces the villus height (Nousiainen, 1991). Absorbed ammonia must be excreted as urea with an energy cost of approximately 7% of the total energy expenditure in monogastric as well as in ruminant animals (Eisemann and Nienaber, 1990) influencing the metabolism and resulting in reduced animal performance (Visek, 1984).

The major benefits derived from the use of subtherapeutic doses of antibiotics in animal feeding were: disease prevention, improved feed utilization, and increased growth rate. These effects were more evident in younger, stressed animals (Hays, 1969) and where management and hygiene conditions were worse. Especially in pigs, most feed antibiotics were used in newly weaned piglets, a critical time for infection in these young animals, and only to a lesser extent in older pigs being raised for slaughter, where their use was generally regarded as

unnecessary and not cost effective.

Feed antibiotics occasionally have been shown to reduce the number of bacteria present in the gut (Jensen, 1988) but more often to have little effect on total counts of viable bacteria.

Several researchers observed that animals receiving diets added with antibiotics showed similarities with germ-free animals (Stutz *et al*., 1983; Nousiainen, 1991): reduced gut weight and length, thinner intestinal wall, and reduced cell turnover in the gut mucosa. Although a final explanation for these effects has not been found yet, they surely are related to changes in the composition of the intestinal microflora. The mechanism by which the antibiotic growth promoters act is not known by certainty, but several hypothesis have been made: 1) a reduction in the thickness of the intestinal mucosa and, as a consequence, a more efficient absorption of nutrients; 2) energy and nutrients are spared because of a reduction of competitors microorganisms; 3) the production of discrete lesions in the cell wall of enteric bacteria and the reduction of microorganism responsible for intestinal disorders; 4) a reduction in amounts of bacterial toxins and toxic metabolites produced in the intestine; 5) an increase in intestinal alkaline phosphatase levels; 6) a decrease in the level of production of intestinal ammonia; 7) a reduction of microbial deconjugation of bile salts. (Jensen, 1998).

Since the introduction of AGP in animal husbandry, an increasing concern on the widespread of antibiotic resistant microorganisms conducted Europe toward the ban of AGP, as of January 1st, 2006; this led researchers to find alternative molecules to be used in animal production which act antibiotic-like, whithout being a threat to human

health.

2.3. Strategies to reduce the impact of post weaning syndrome (PWS)

Weaning is a very crucial moment in the growth of pigs; there are many factors which contribute to the rise of gut infections, which have a high economic impact in pigs husbandry, especially after the removal of antibiotic growth promoters (AGP). These infections are commonly called Post Weaning Syndrome (PWS) or Post Weaning Colibacillosis (PWC) (Pluske et al., 2002) since it seems to be *E. coli* that is main cause of such syndrome. Even if it has been demonstrated that *E. coli* is necessary for diarrhea outbreaks, PWS is nowadays regarded as a multifactorial disease (Dirkzwager et al., 2005; Pluske et al., 2002) which needs many cofactors playing important roles in predisposing to its development. Among these "causative agents", we can list environmental factors, such as the separation from the mother, the regrouping of animals, the exposure to a new antigenic environment, and the "cold - stress" due to the lack of thermoregulation capacity (Wathes et al., 1989). All those factors contribute to make piglets stressed with a consequent higher production of cortisol which, via the sympathetic nervous system, alters the intestinal transit time and causes immune suppression (Pluske et al., 2002). Furthermore, the piglets, during weaning, lose any passive antibody protection provided from the sow's milk. Anorexia and feed refusal are important consequences of stressful weaning and contribute in worsening the yet dramatic situation. In addition, there are other factors, such as physiological factors as the passage from liquid to solid feed. The possibilities for maximizing sow productivity and the need to more efficiently utilize the expensive farrowing facilities have led pig producers to wean piglets at three to four weeks of age. At that time, the

gastrointestinal (GI) tract and its digestive enzymes set are not fully developed (Marion et al., 2002), the bacterial colonization is still transient and the piglets result more exposed and sensitive to anti-nutritional factors present in a vegetable diet, being therefore more susceptible to mal-absorption. The first consequence is a larger amount of undigested feed in the intestine, which represents a good substrate for microorganism overgrowth (Dirkzwager et al., 2005; Hopwood et al., 2003).

Acid secretion in young pigs does not reach appreciable levels until 3 to 4 weeks after weaning (Cranwell and Moughan, 1989). The suckling pig employs several strategies to overcome the limitation of insufficient acid secretion and these have been discussed by Easter (1988). The primary strategy involves the conversion of lactose in sow's milk to lactic acid by Lactobacilli bacteria residing in the stomach. Secondly, the nursing pig reduces the need for transitory secretion of copious amounts of acid by frequent ingestion of small meals. Finally, diets are known to differ widely in acid-buffering capacity: this capacity is lowest in cereals and cereal by-products, intermediate or high in protein feedstuffs and very high in mineral sources, except in dicalcium and monosodium phosphates (Jasaitis et al., 1987).

The failure to maintain a low gastric pH has major implications for the performance of the early-weaned pig. First, an elevated pH would cause a

reduction in the activation of pepsinogen which occurs rapidly at pH 2 and very slowly at pH 4 (Taylor, 1962). The pepsins have two optimal pH levels, 2 and 3.5, and their activity declines above 3.6 with no activity at pH > 6.0 (Taylor, 1959). As a result, feed proteins may enter the small intestine essentially intact with an eventual reduction in efficiency of

protein digestion. The end-products of pepsin digestion also stimulate the secretion of pancreatic proteolytic enzymes (Rerat, 1981). Furthermore, acid from the stomach is the primary stimulant for pancreatic secretion of bicarbonate (Kidder and Manners, 1978). In addition, acid leaving the stomach plays a role in the feedback mechanism in the regulation of gastric emptying, thus, decreasing the digesta load on the small intestine. Secondly, an acid gastric condition is believed to have pronounced bactericidal properties for certain microorganisms, in particular for the Coliforms (Sissons, 1989). Viable microorganisms entering the digestive tract via the mouth are unable to pass through the acidic conditions of the stomach and successfully colonize the small intestine. A rise in gastric pH would, therefore, allow increased proliferation of Escherichia coli (Smith and Jones, 1963) which has been associated with scours and increased mortality (Thomlinson and Lawrence, 1981). Furthermore, evidence suggests that proliferation of Coliforms in the stomach may lead to further diminution of gastric acid secretion due to the release of a bacterial polysaccharide with an inhibitory effect on acid secretion (Baume et al., 1967; Wyllie et al., 1967).

All the above cited circumstances, e.g. stress, anorexia, and physiology, cause great damages to the intestinal mucosa, which undergoes to several structural changes such as the reduction of villi height and an increase in crypts depth (Pluske et al., 1996). The reduction of the intestinal absorptive area and the appearance of a less mature enterocyte population help to explain the increased susceptibility of the pig to diarrhea. In fact, in the small intestine, nutrients, electrolytes, and water are absorbed by villus enterocytes, whereas electrolytes and water are secreted in crypt cells (Powell, 1987). Because shorter villi and deeper

crypts have fewer absorptive and more secretory cells, absorption might be poorer and secretion increased. A heat-stable toxin (toxin b) produced by some Escherichia coli strains is known to be villus shortening (Whipp et al., 1986).

During a study on weaned pigs, Nabuurs et al. (1993) recovered an Escherichia coli strain that produced heat-stable toxin b from all the litters affected by diarrhoea.

Genetic also could play a key role in PWC syndrome, since it has been studied that certain pigs do not express glycoproteic receptors for *E. coli* K88 fimbriae in the brush border of cells lining the intestinal villi in the small intestine (Hampson, 1994).

In this context, practical feeding and management strategies can be adopted in order to ameliorate post weaning conditions and piglets health status.

2.3.1 Improvement of gut development

Stimulating the feed intake before and after weaning by supplying a palatable diet during the first 5-10 days is a very important tool in help a normal gut development; the light management can significantly influence this parameter, since during the dark period the ingestion is depressed (Dirkzwager et al., 2005). Another strategy could be to supply the diet with substrates like butyrate, which is known to be an intestinal mucosa growth factor (Piva et al., 2002).

2.3.2. Inhibition of pathogenic bacteria

Adding organic acids to the diet cause the pH decline in the gut and may inhibit the growth of E. coli pH-sensitive and improve stomach

functionality by decreasing its empting rate, while supplementing the diet with essential oils or plant derivatives, which have well known antimicrobial properties (Burt, 2004) can reduce intestinal pathogens shedding.

2.3.3. Improvement of feed digestion

The right ingredient choices and highly digestible nutrient sources are a very important tool ain controlling PWS associated intestinal microbial proliferation. Numerous studies investigated the correlation between the inclusion of soluble NSP in piglets starter diets and the proliferation of *E. coli.* Feeding piglets with pearl barley meal increased intestinal viscosity and altered microbial fermentation, helping *E. coli.* proliferation both in small and large intestines as demonstrated by Mc Donald et al. (2001). These data suggests that the presence of soluble NSP in weaned diets is an undesirable effect, which can be overcome by selecting cereals low in NSP content or adding exogenous enzymes, such as β-glucanases or xylanases to increase soluble NSP digestibility.

The source of proteins, such as vegetable or animal proteins, is another very important aspect to be evaluated, since it has been demonstrated that soybean meal is rich of anti-nutritional factors that reduce protein digestibility and induce mucosal damages, especially in highly susceptible weaning pigs (Miller et al., 1984). Proteins of animal origins such as milk whey or fish meal proteins are therefore preferable to soybean proteins. Hall and Byrne (1989) observed villus stunting, loss of activity of lactase and sucrase from the mucosa and reduced live weight gain in weaning pigs when the diet was changed from milk to a dry pelleted meal feed containing milk, soya and cereal proteins without

any interactions with microbial pathogens. They supposed that the intestinal damage detected could be the result of antibody-mediated damage induced by antibodies to soy proteins in the meal.

Nutritionists have attempted to overcome the digestive inefficiency of early weaned pigs by incorporating milk products in cereal and oilseed meal based diets. Milk products of high quality have consistently improved post-weaning growth performances (Pals and Ewan, 1978; Graham et al., 1981; Cera et al., 1988). These beneficial effects arise from their high content of lactose (Mahan, 1992) which is not only more digestible than the complex carbohydrates from plant sources, but is also fermented to lactic acid to provide gastric activity (Wilson and Leibholz, 1981) and to act as a non-digestible oligosaccharide (Piva et al., 1998).

Although the definition of probiotics presents considerable problems and has varied over the years, the final aim of their aim of their application is that probiotics should have beneficial effects on animals by the establishment of an optimum balance of microbes in the alimentary tract. Salminen et al. (1996) have summarized the most important functional effects of probiotics: these include aspects such as immune modulation and strengthening the gut mucosal barrier, due to: 1) gut microflora modification; 2) adherence to the intestinal mucosa with capacity to prevent pathogen adherence or pathogen activation; 3) modification of dietary proteins by the intestinal microflora; 4) modification of bacterial enzyme capacity, especially of those suggested to be related to tumor induction; 5) influence on gut mucosal permeability.

3. Enzymes as an alternative of antibiotics in swine feed

In recent years, there has been considerable interest in finding or developing alternatives to antibiotic growth promoters. Many types have been examined: probiotics (bacterial cultures), oligosaccharides and yeast, other carbohydrates, plant extracts and nucleosides. Table 1. shows the efficacy and potential for developing alternatives to replace antibiotics. In general, all these products have produced variable results in pig production, best results being obtained with the probiotics, organic acids and enzymes.

Table 1. The efficacy and potential for developing alternative additives and strategies to replace the role of antibiotic feed additives in pig production

Alternative feed additives	Efficacy*	Potential for development*
Antibiotics	+++++	0
Zinc oxide	++++	0
Copper sulphate	+++	0
Organic acid	+	0
Enzymes	+++	+++
Probiotics	+	+
Fermentable substrate (Prebiotics)	++	+++
Herb extracts	?	+
Soya isolates	+	+
Immunoglobulins	++	?

*Efficacy and development based on a subjective score 0 (zero) to ++++ (very high), or ? (unknown)

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3.1. Non-starchpolysaccharides (NSP)

3.1.1. Definition and classification

Carbohydrates can also be classified physiologically (Table 2) by dividing them into digestible and indigestible compounds. Digestible carbohydrates, including amylose and amylopectin, are easily hydrolyzed by mammalian enzymes in the small intestine yielding glucose that is mainly used for energy.

NSP, resistant starches, and certain oligosaccharides, the main forms of undigestible carbohydrates are not digested in the small intestine because pigs lack the enzymes necessary to cleave each compound's specific bonds. Indigestible carbohydrates pass into the large intestine essentially intact where they become possible substrates for microbial growth. However, not all undigestible carbohydrates are fermentable leading to the further classification into fermentable and non-fermentable categories.

The NSPs represent a group of heterogenous compounds which greatly differ in chemical composition and physical characteristics both within and between plant varieties. In general, nine of monosaccharides are known to be major building blocks of NSP. These include the pentoses (arabinose and xylose), hexoses (glucose, mannose, and galactose), the 6-deoxyhexoses (rhamnose and fucose) and hexauronic acids (galcturonic acid and glucuronic acid). These sugars are joined primarily by glycosidic bond. The NSPs may comprise up to 90% of the cell wall of plants (Selvendran and Robertson, 1990), and main matrix of NSPs in plant cell walls include cellulose, hemicelluloses, and pectins.

Cellulose fibers in the plant cell wall are interconnected with

hemicellulose molecules that are hydrogen bonded to the surface of the cellulose microfibrils. This cellulose/ hemicellulose complex is embedded in a matrix of pectin. The composition of the plant cell wall varies considerably among plant types (i.e., dicot vs. monocot) and within species of plants. The main load-bearing component of the wall is cellulose, which surrounds the cell by forming long fibrils composed of 30 to 36 chains of β-1,4-linked glucose that are hydrogen bonded into a single strand (Vorwerk et. al., 2004). Multiple chains of cellulose form strong hydrogen bonds linking together to form microfibrils. Multiple microfibrils also interconnect via hydrogen bonding to provide the rigidity of the plant cell wall. These tightly packed fibrils are insoluble in water and are therefore difficult to digest. Cellulose is the most prevalent plant material in the world (Bhat, 2001) and is estimated to be produced at over 100 billion metric tons per year (Ryu and Mandels, 1980).

Hemicelluloses are a large group of polysaccharides found in the cell walls of all land based plants. They are involved in the structure of the cell wall and covalently link cellulose to lignin. Hemicelluloses rank second to cellulose in total abundance in plant materials (Bhat, 2001). One-fourth to one-third of all plant material is comprised of hemicellulose and the amount varies according to the particular species of plant (Cosgrove, 1997). Hemicelluloses are low molecular weight, branched polysaccharide chains with a β-1,4-linked xylopyranosyl backbone. The number, proportion and linkage of the sugar units vary among the different hemicelluloses. The hemicelluloses are classified into 5 groups, xylans, glucomannans, arabinans, galactans, and glucans. These groups are based on the main sugar residue in the backbone. The typical sugars of hemicellulose are D-xylose, L-arabinose, D-glucose, D-galactose,

D-mannose, Dglucuronic acid, 4-O-methyl-D-glucuronic acid, D-galacturonic acid and to a lesser extent L-rhamnose, L-fucose and various O-methlyated neutral sugars (Sun et al., 2004). The most abundant hemicelluloses are xylans (found in wheat, rye and triticale), which are present in all terrestrial plants and comprise up to 30% of all the cell wall material of annual plants (Sun et al., 2004). Another major hemicellulose, β-glucans, is a mixture of β-1,4 and β-1,3-glucose, found in the cell wall of oats and barley.

The term, pectin, refers to galacturonic acid-containing carbohydrates of the cell wall. Pectic polysaccharides are composed of a chain of 1,4-linked α-d-galactosyluronic acid with the occasional α -d-rhamnose in the backbone and side chains of primarily galactans and arabinans (Ridley et al., 2001).

Table 2. Classification of carbohydrates (Knudsen, 1997)

Category	Monomeric residues	Sources
Non-starch polysaccharides (NSP)		
Cell Wall NSP		
Cellulose	Glucose	Most feedstuff
Mixed linked β-glucans	Glucose	Barley, oats, rye
Arabinoxylans	Xylose, arabinose	Rye, wheat, barley
Arabinogalactans	Galactose, arabinose	Cereal by-products
Xyloglucans	Glucose, xylose	Cereal flours
Rhamnogalacturans	Uronic acids, rhamnose	Hulls of peas
Galactans	Galactose	Soy bean meal, beet pulp
Non-Cell wall NSP		
Fructans	Fructose	Rye
Mannans	Mannose	Coconut cake, palm cake
Pectins	Uronicacids, rhamnose	Beet pulp
Galactomannans	Galactose, mannose	Guar gum
Non-digestible oligosaccharides (NDO)		
Alpha-Galacato-oligosaccharides	Galactose, glucose	Soy bean meal, peas
Fructo-oligosaccharides	Fructose	Cereals, feed additives
Transgalacto-oligosaccharides	Galcatose, glucose	Whey, milk products
Resistant starch (RS)		
Physical inaccessible starch	Glucose	Peas, faba beans
Native starch	Glucose	Potatoes
Retrograded starch	Glucose	Heat-treated starch products

3.1.2. NSP in feed ingredients

The major polysaccharides present in grains and legumes are presented (as average values) in Table 3. However, from a physiological perspective, it is important to consider also the variation that exists within a particular cereal because this may determine some of the physicochemical properties of the grain in vivo that, in turn, are likely to have an effect on digestibility and performance.

The NSP in cereal grains are composed predominantly of

arabinoxylans (pentosans), β-glucans and cellulose. Only small amounts of pectic polysaccharides are found in the stems and leaves of cereals. With the exception of rice (Shibuya and Nakane 1984), there is no evidence that they occur in the maternal tissues of cereals. Corn and sorghum contain very low levels of NSP, whereas wheat, rye and triticale contain substantial amounts of both soluble and insoluble NSP. The main soluble NSP in these grains are arabinoxylans, whereas barley and oats β-glucans.

Legumes, such as peas, beans, soybean and lupins, and oilseeds and (or) oilseed meals, such as canola (rapeseed meal), are commonly included in weaner pig diets. These feedstuffs contain considerable quantities of NSP and may themselves exert anti-nutritive effects. Whilst soybean meal is recognized as virtually the 'gold standard' in vegetable protein sources available for pig diets. Most of all, the oilseeds possess a certain amount of anti-nutritional factors, and soybeans are no exception.

Raffinose and stachyose are two types of short-chained carbohydrates that make up about 5-7% of the soybean. These oligosaccharides are not digested and they cause digestive disturbances and depressed growth in early-weaned pigs. The oligosaccharides can be removed by special processing, resulting in a product called soy protein concentrate.

Table 3. Typical NSP contents (g/ kg dry matter) and major polysaccharides in grains and legumes.

Ingredient	Soluble NSP	Insoluble NSP	Total NSP	Major NSP
Wheat[a]	25	94	119	Arabinoxylan
Barley (hulled)[b]	45	122	167	β-Glucan
Barley(hull-less)[a]	50	74	124	
Rye[a]	42	110	152	Arabinoxylan
Oat(hulled)[a]	40	192	232	β-Glucan
Oat(hull-less)[a]	55	63	116	
Soybean[c]	27	16	192	Galacturonans, arabinanas and galactose
Pea[c]	25	322	347	Rhamnogalacturonan, glucan
Lupin[c]	46	320	366	Rhamnogalacturonan, arabinose and galactose
Lupin kernel[d]	27	218	245	

[a]Bach Knudsen (1997); [b]Englyst (1989); [c]Choct (1997); [d]Annison *et al*. (1996).

3.1.3. Physiological effect of NSP in swine feed

Effects of NSP on gut physiology

Numerous workers have shown that soluble NSP suppresses the activity of certain pancreatic enzymes *in vitro*, namely, amylase, lipase, trypsin and chymotrypsin (Mosenthin et al., 1999). An in vitro human study found that cellulose and xylan reduced the activity of amylase, lipase, trypsin and chymotrypsin to less than half their original activity (Dunaif and Schneeman, 1981). The reduction in enzyme activity was due to non-specific binding of the enzymes by the NSP polymers. However, the inhibitory effects of NSP on the activity of the intestinal enzymes may not have significant effects on the digestibility of food in the small intestine, because of the very large excess (‘spare capacity’) of enzyme activity present in pancreatic secretions (Selvendran et al., 1987).

To support this notion, Lizardo et al. (1997) fed 25-day-old weaned piglets one of four wheat-based diets that contained 0 or 120 g sugar beet pulp kg^{-1} as the major source of NSP, with additional protein being supplied by either soybean meal or soluble fish protein concentrate. These authors showed that when pigs were euthanized at 56 days of age, feeding 120 g sugar beet pulp kg^{-1} sustained the activity of intestinal and pancreatic enzymes, though no data showing digestibility were presented. Similarly, Jensen et al. (1998) found no differences in pancreatic enzyme activities when 28-day-old weaned pigs were fed barley-based diets with or without β-glucanase addition.

Larger amounts of water are found within the gut when the content of dietary NSP is increased, due to the hydrophilic nature of NSP and increased endogenous secretions. For example, wheat bran increased secretion of pancreatic juice by 115%, protein by 40%, chymotrypsin by 59%, trypsin by 53%, lipase by 78% and amylase by 70% (Low, 1989). Moreover, NSPs elevate the secretory output from the salivary glands, stomach, liver, pancreas and intestinal wall. These cause an increased excretion of water, proteins, lipid and electrolytes. In particular, soluble NSPs increase the volume of food in the gut by entrapping a large volume of water. This process will increase intestinal secretion, because the greater volume of food elevates intestinal secretions (Low, 1989).Prolonged intake of soluble NSP shows significant adaptive changes in the digestive system. The changes in the gut are characterized by enlargement of the digestive organs and increased secretion of digestive juices (Low, 1989; Choct, 1997).

Apart from the above direct effects of NSP on gut physiology, soluble NSP may also modify gut physiology by interacting with

microflora in the small and large intestine of pigs (Langhout et al., 2000). Insoluble NSP may diminish the overall bacterial activity in the intestinal tract by decreasing the time available for fermentation in the gut; also, bacteria may adhere to the insoluble NSP structure (Smits and Annison, 1996). However, viscous soluble NSPs significantly elevate fermentation in the terminal part of the small intestine. Since soluble NSPs increase the average retention time of digesta in the gastrointestinal tract (Smits and Annison, 1996; Choct, 1997), an excellent environment is created for anaerobic microflora due to decreased oxygen tension. Increased retention time may increase the amount of undigested material in the small intestine, which gives the anaerobic microflora more time and substrates to colonize the proximal small intestine.

Consequently, greater retention time provides more chances for enhanced bacteria adhesion to the mucosal surface which is an essential process in a number of bacterial infections. Production of toxins and deconjugation of bile salts which are essential for the digestion of fat may be increased by proliferation of some anaerobic organisms because most of the soluble NSP sources are fermentable (Smits and Annison, 1996; Choct, 1997).

An increase in bacterial activity in the small intestine may cause a systemic effect on the gut secretions and morphology of the small intestine. As a result, poor digestibility of nutrients may result from reduced nutrient absorption through the affected gut walls. In addition, digestible carbohydrates such as starch and glucose are converted through microbial action to volatile fatty acids which represents inefficiency of nutrient utilization by monogastric animals (Smits and Annison, 1996; Choct, 1997).

Effects of NSP on nutrient digestion

Non-starch polysaccharides have been recognized as 'anti-nutritive' due to their negative influence on digestion and absorption of starch, protein and lipid in the gut of some monogastric animals, particularly the broiler chicken. The soluble fractions are considered of major importance in determining the nutritive value of feedstuffs for monogastric animals.

The soluble NSP may increase digesta viscosity, and the increased bulk and viscosity of the intestinal contents will decrease the rate of diffusion of substrates and digestive enzymes (Ikegami et al., 1990; Classen and Bedford, 1991). In other studies, the convective transport of glucose was impaired in an in vitro viscous environment (Smits and Annison, 1996) and an arabinoxylan-rich extract from rye decreased the rate of dialysis of glucose (Fengler and Marquardt, 1988).

Supplementation with appropriate enzymes has been shown to increase energy utilization in wheat-fed broiler chickens (Annison, 1992) and in pigs (Graham et al., 1989; Baidoo et al., 1998). In addition, NSP depressed protein digestion in broiler chickens (Choct and Annison, 1992) and in pigs (Bedford et al., 1992; Baidoo et al., 1998).

Numerous authors have demonstrated that endogenous nitrogen loss, at least in part from mucins, is significantly increased with increased digesta viscosity (Larsen et al., 1993, 1994). Furthermore, high viscosity stimulates epithelial cell proliferation and may contribute to some loss of epithelial cells when an animal is given soluble NSP (Gee et al., 1996). It was found that soluble NSP with high water-holding capacities disrupted protein digestion and absorption in pigs, while insoluble NSP with high water-holding capacity had no influence on protein digestion

and absorption (Leterme et al., 1998). Using 11-week-old pigs (14 kg) fed different sources of neutral detergent fiber (NDF), Schulze et al. (1995) determined that NDF caused increases in ileal nitrogen flow (reduced digestibility) as a result of increases in both endogenous and undigested dietary ileal nitrogen losses.

These authors recommended that diets containing in excess of 200 g NDF kg^{-1} should be supplemented with approximately 10 g ileal digestible protein per kilogram of diet to compensate for these losses.

3.2 Roles of enzymes in animal nutrition

Enzymes are amino acid chains that reduce the amount of energy required for chemical reactions that normally would not take place under physiological conditions. They catalyze reactions by binding to their specific substrates causing a conformational change in the compounds resulting in end product formation. Enzymes are specific catalysts and their three dimensional structure determines activity and substrate. Any change in structure alters the enzyme specificity making environmental conditions, such as pH and temperature, important in maintaining enzymatic activity. Enzymatic activity in most biological systems tends to be the highest at a combination of neutral pH and mild temperatures.

The rationale for adding exogenous enzymes to animal diets includes four factors (Sheppy, 2001):

1. To facilitate the break down of anti-nutritional factors present in many feed ingredients
2. To increase the availability of starches, proteins and minerals that are either enclosed within the fiber rich cell wall or bound in a

form that is indigestible (i.e.,, phosphorus in phytic acid)

3. To break down specific chemical bonds in raw materials that are not usually broken down by the animals' secretory enzymes, thus releasing more nutrients for further enzymatic degradation and absorption.

4. To supplement endogenous enzymes produced during times of underproduction (i.e.,, young or sick animals with improper or immature digestive function)

Enzymes used in animal nutrition are grouped into common functional capacities: fiber-degrading, protein-degrading, starch-degrading, and phytic acid-degrading (Sheppy, 2001). Currently, many of feed enzymes have been developed and commercialized in the feed industry (Table 4).

Table 4. Feed enzymes in use for degrading NSP.

Enzyme	Action	Substrate	Type of feed	Expected benefits
ß-glucanase	ß-glucans to oligosaccharides and glucose	Barley, oats and rye based diets	Poultry and pigs diets	Reduction of droppings, improved feed utilization
Cellulases	Cellulose to low molecular	High fiber diets	Poor-grade forages	Improved energy Availability
Xylanase	Arabinoxylans to low molecular weight sugars	Rye, barley and wheat	Pig and Poultry diets	Improved litter quality and feed utilization
α-galactosidase	Degrades oligosaccharides and ANFs	Soybean and other legumes	Pig and poultry diets	Improved energy availability, reduced scours

(Ogden, 1995; modified from Rotter et al., 1989; Cowan, 1992)

Xylanases (pentosanase) and β-glucanases are the most commonly used carbohydrases in swine diets. Pigs lack the specific enzymes needed to degrade cell-wall polysaccharides (mainly β-glucans and arabinoxylans) prior to their fermentation in the cecum and large intestine. Thacker et al. (1991) added xylanase (1,625 U/kg) to a rye based diet and demonstrated no effect on the growth performance or carcass characteristics of grower-finisher pigs.

Choct et al. (2004) evaluated the use of xylanase (1000 U/kg) addition, steeping, or the combination of the two on the performance of 27-d old pigs fed a wheat, lupin, rice, canola and SBM based starter diet using a liquid feeding system. They reported that enzyme addition resulted in increases in daily feed intake and average daily gain but resulted in a reduction in DE content of the diets.Diets that include barley, rye, wheat, triticale or oats, contain large amounts of β-glucans. β-Glucans are not well digested by non-ruminant animals and may reduce the digestion of other nutrients by increasing the viscosity of the chyme. The enzyme β -glucanase breaks the β-1,3 linkages in the glucose polymer. β-Glucanase has been widely adopted in the poultry industry allowing for the large scale inclusion of barley as a major feedstuff. However, significant disappearance of β-glucans have been shown in swine small intestine (Li et al., 1996) resulting in swine being less susceptible to digesta viscosity problems than other nonruminant animals (Danike et al., 1999).

The effect of β-glucanase on nutrient digestibility was evaluated by Li et al. (1996) in young pigs fed hulless barley-SBM or wheat-SBM diets supplemented with β-glucanase at 2000 Units/ kg feed. They demonstrated improvements in amino acid digestibility of diets

supplemented with a β-glucanase. However, this mixture contained β -glucanase and cellulase (β-glucosidase) activity possibly confounding the effects of β-glucanase alone.

Yin et al., (2001) supplemented xylanase, β-glucanase, or a combination of xylanase, β-glucanase and protease to diets containing various varieties of hulless barley and fed them to young pigs evaluating ileal nutrient digestibilities. Enzyme supplementation resulted in improvements in amino acid (AA), crude protein (CP), NDF, NSP and energy digestibilities, with the β-glucanase treatment providing the greatest effect. Xylanase addition alone resulted in slight improvements in the digestibility of various amino acids and the cocktail did not enhance digestibility over β-glucanase supplementation alone.

Currently there have been no demonstrated benefits of adding carbohydrase enzymes to swine diets. Responses to carbohydrase supplementation in swine have been variable with experiments demonstrating no improvement (Kim et al., 2003; van der Meulen et al., 2001; Diebold et al., 2004), mixed improvements (Mavromichalis et al., 2000), and small improvements (Barrera et al., 2004; Omogbenigun et al., 2004) in digestibility and performance characteristics.

4. Dietary supplementation of β-mannanase

β-Mannanases are common constituents of the plant cell-wall-degrading arsenals of various bacteria (Tamaru et al., 1995), fungi (Kurakake and Komaki, 2001), plants and animals (McCleary, 1988). These enzymes refer to the enzymes capable of hydrolyzing mannopyranosil linkages, namely: pure mannan, galactomannan, glucomannan and galactoglucomannan.

Three types of β-Mannanases exist to effectively hydrolyze the linkages of either pure mannan or galactomannan; endo-mannanase (EC 3.2.1.25), exo-mannanase (EC 3.2.1.78) and α-galactosidase (EC 3.2.1.22). Endo-mannanase (mannan mannohydrolase) is only capable of breakdown the mannan and galactomannan backbone to yield mainly mannotriose, mannobiose and a small amount of mannose (Sabini et al, 2000).

To further hydrolyse the soluble sugars, exo-mannanase is more effective. Unfortunately the availability of this particular enzyme in commercial market is scarce and expensive and thus, this enzyme is only used for scientific purposes rather than for commercial purposes.

4.1 Mannan

Mannan is a major constituent of hemicellulose where it exists in a variety of forms, including linear mannan, glucomannan, galactomannan, or glucogalactomannan (Fig. 1.). Each of these polymers comprises a b-1,4-linked backbone of mannose residues that may be substituted up to 33% (or up to 50% in hardwoods) with glucose residues. In the case of galactomannans or glucogalactomannans, galactose residues form α -1,6-linkages to the mannan backbone (Moreira and Filho, 2008).

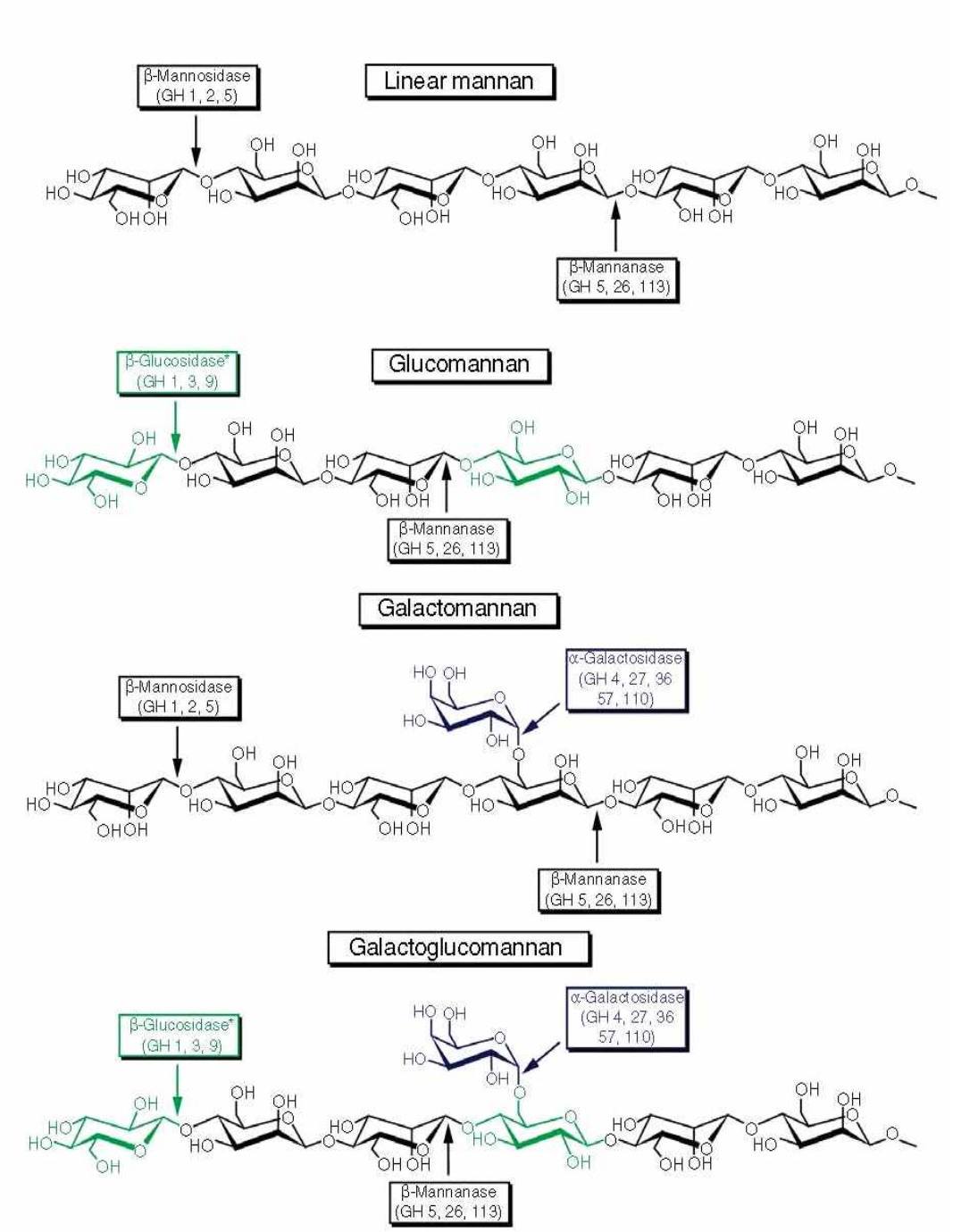

FIGURE 1. Enzymatic activities associated with hydrolysis of various mannans. There are four different types of mannan: linear mannan, glucomannan, galactomannan, and galactoglucomannan. (Carl J. Y et al., 2010)

Mannan is easily found in grain or oilseed feed ingredients, such as SBM, copra meal, palm kernel meal, soyhulls, sesame meals (Dierick, 1989) and in corn-distillers dried grain with soluble (DDGS) (Tucker et al., 2004).

Studies (Peng et al., 1991; Zhang and Tizzard, 1996; Ross et al., 2002) have shown that β-mannan is capable of stimulating the innate immune system and is thus, potentially capable of stimulating a nonproductive energy draining innate immune response. This results in an increase in proliferation of macrophages and monocytes, and increased cytokine production, leading to an increased severity of disease symptoms and a decrease in the efficiency of nutrient use. Moreover, dendritic cells, which have many surface receptors including mannan binding sites (van Kooyk and Geijtenbeek, 2003), are known to open the tight junction between epithelial cells and then extend into the intestinal lumen to sample lumen antigens (Kraehenbuhl and Corbett, 2004). This is part of the monitoring system that gut immunity deploys to check the micro-environment for changes in the intestinal lumen. This monitoring ability may provide a mechanism for contact between immune cells and β -mannan from soy in the lumen.

Because of the almost universal use of SBM and full fat soy as protein sources in swine feeds, β-mannan is present in the majority of swine diets currently used around the world. However, β-mannan has known to be anti-nutritional factors due to their highly viscous properties, which influences negatively on animal performance (Anderson and Warnick, 1964; Jackson et al., 1999) and thus, it regarded as anti-nutritional factors.

Several studies reported the negative effects of mannans in

monogastric animal feed. Leeds et al. (1980) demonstrated that β-galactomannan disturbs glucose metabolism and insulin secretion rates in pigs. Dale, (1997) reported the high viscosity of β-mannans probably decrease the efficiency of carbohydrate utilization of monogastric animals by blocking key sites on the intestinal surface. In broilers, increasing mannan inclusion rate of 2 to 4% in feed severely slowed growth and decreased feed efficiency (Couch et el, 1967; Ray et al, 1982; Verma and McNab, 1982).

Mannans also interferes with insulin secretion and glucose absorption. Rainbird et al., (1984) suggested that increasing mannan intake has been shown to reduce the rate of glucose absorption from 74.2% to 41.4% and water absorption and consequently diminish carbohydrate metabolism by interfering with insulin secretion and IGF (insulin-like growth factor) production (Nune and Malmhof, 1992). Table 5. showed the feed ingredient which containing mannan.

Table 5. The percentage of mannan in the feed ingredients

Ingredient	Mannan % (DM)
Palm kernel meal	30 ~ 35
Copra meal	25 ~ 30
Guar meal	12 ~ 17
Soy hulls	6 ~ 10
Sesame meal	2.8 ~ 3.5
SBM	1.2 ~ 1.6
Lupine seed meal	0.4 ~ 1.0
Barley	0.49
Wheat	0.10
Corn	0.09
wheat bran	0.07

(Dierick, 1998)

4.2 Mode of action of β-Mannanase

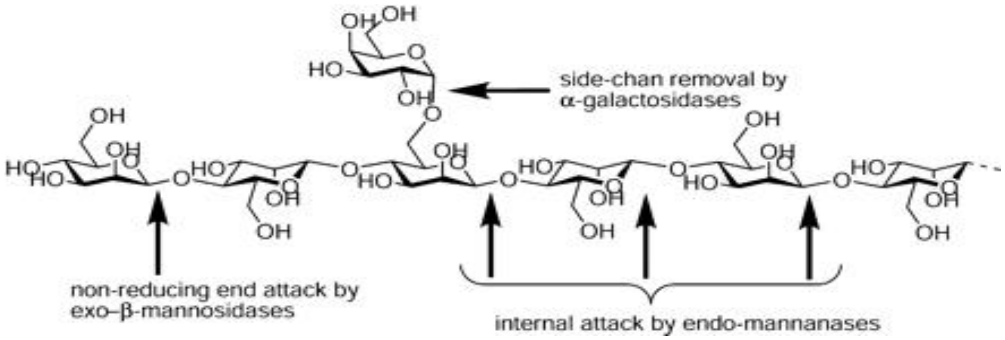

Figure 2. Schematic representation of galactomannan and the enzymes involved in its degradation (Dias et al., 2004)

Figure 2. shows structures if galactomannan and enzyme acting points. The majority of β-Mannanase mechanism is complete decomposition and conversion of the mannan to mannose or MOS (Mannan-oligosaccharides).

MOS is oligosaccharide which is composed of mannose. Oligosaccharide is a group of carbohydrates containing 2~10 sugar units and its structure is linear or branched linkage of carbohydrates. Many studies reported positive effect of MOS in pig's gut microflora, immune function, and inhibited colonization of the digestive tract by unfavorable microorganisms in livestock species, consequently promote overall health and growth by decreasing pathogenic bacteria (Roseboom et al., 2005) and immune modulation (Spring and Privulescu, 1998).

Mannose is a sugar monomer of the hexose series of mannan fraction. It concerned in a metabolic transformations from glycoproteins and glycolipids to fucose. Mannose plays an important role in glycoprotein biosynthesis because of the identification of mannose-specific receptors in several mammalian cell type. Not so

much of studies have conducted about mannose bioavailability, absorption, fate or incorporation in higher animals. Wood and Cahil (1963) concluded that oral mannose absorption efficiency was not good, but infused mannose was well tolerated and cleared normally.

Other mode of action of β-Mannanase is reduction in innate immune stimulation associated with a reduction in th β-Mannan content of substrate entering the intestinal tract (Jackson et al., 2004). β -Mannanase also crossing the intestinal mucosa are potent stimulators of the innate immune system, resulting in increased proliferation of macrophages and monocytes and resultant cytokine production. These result in exacerbated disease symptoms and reduced nutrient use, which has been observed using galactomannans derived from fungi (Ross et al., 2002).

4.3 Application to mono-gastric animals

Application to poultry diet, β-mannanase improve feed efficiency of broilers fed corn-SBM diets (Ward and Fodge, 1996). β-mannanase supplementation diet containing SBM improved growth and feed efficiency in broilers and turkeys when 3% up of β-mannanase inclusion rate (James et al., 1998; McNaughton et al., 1998). The supplementation of β-mannanase to broiler diets containing 2% guar gum showed negative effects on growth performance (Daskiran and Teeter, 2001). Anderson et al (2001) reported that β-mannanase was able to improve feed efficiency and increased uniformity of body weight in broiler.

Jackson et al. (1999) reported that β-mannanase was capable of increasing egg weight in commercial layers at early stages of

production and increasing egg production, particularly delaying the post peak decline in productivity. Supplementation β-mannanase showed significant improvement in overall average feed efficiency of leghorns fed the low energy diet in second-cycle hens, which indicated that β -mannanase improved energy utilization of corn-SBM based layer diets. This result means the potential to reduce the cost of layer diets containing mannan (Wu et al. 2005).

Application to swine diet, Pettey et al. (2002) showed supplementation to corn-SBM based diet resulted in the similar growth performance of weanling and growing-finishing pigs with the high ME treatment by 100 kcal/kg or 2% oil supplementation. Radcliffe et al. (1999) reported that β-mannanase supplementation to corn-SBM based feed improving apparent energy digestibility in swine and Chen et al. (1998) reported that containing β-mannanase in corn-SBM based diet improved body weight gain and feed efficiency of finishing pigs. Hahn et al. (1995) demonstrated improvements in feed efficiency and lean gain in growing-finishing pig.

Ⅲ. Effect of β-mannanase (Hemicell-HT®) Supplementation, as an Alternative toward Antibiotics, on Growth Performance and Intestinal Integrity in Weaning Pigs

Abstract: This experiment was conducted to investigate the effects of supplementation of Hemicell-HT® on growth performance, nutrient digestibility and intestinal morphology in weaning pigs. A total of 96 weaning pigs (28 ± 3 old and 6.96 ± 0.70 kg of BW) were allotted into 4 treatments with 6 replicates of 4 piglets per pen in a randomized completely block (RCB) design. Treatments were: 1) NC (negative control) (basal diet), 2) PC (basal diet + 0.1 % antibiotics), 3) A (basal diet + 0.04 % Hemicell-HT®), 4) B (basal diet + 0.06 % Hemicell-HT®). Two phase feeding programs (Phase I for 0 - 2 week and Phase II for 3 - 5 week) were used in this experiment. In feeding trial, PC and B treatments showed significantly higher body weight and ADG. There were no significant differences in the glucose, lactate, triglycerides, (acute phase protein) APP level. In nutrient digestibility, pigs fed 0.06% of Hemicell-HT® showed significantly higher crude fat digestibility. The ratio of villi height and crypt depth in jejunum and ileum was higher when pigs were fed diet 0.06% of Hemicell-HT® treatment diet. In moisture contents of feces, pigs fed diet with antibiotics had significantly lower moisture of feces ($P < 0.05$) but 0.04% Hemicell-HT® treatment showed statistically similar moisture content compared to positive control in all phases. From this study,

Hemicell-HT® instead of dietary antibiotics can be supplemented in weaning pig's diet without growth check or detrimental effects. Consequently, this experiment demonstrated that dietary enzymes, Hemicell-HT® can be supplemented in weaning pig's diet without growth check or detrimental effects.

Keywords : Hemicell-HT®, Weaning pigs, Growth performance, Intestinal morphology, Moisture content of feces

Introduction

Antibiotics have commonly been used as growth stimulants and to treat gastrointestinal infections subtherapeutically (Verstegen and Williams, 2002). However, reducing or banning the use of antibiotics has already become a global trend because of its overuse and side effects. While antibiotics are known to promote growth of livestock, the increasing consumer concerns on antibiotic resistance have put pressure on the producers to reduce the use of these antibiotics. In EU, supplementation of antibiotics in animal feed as AGP was completely banned from January 1st 2006 and Korea also prohibited using antibiotics in swine diet legally from July 1st 2011. Although alternatives toward antibiotics are not clearly established, various unverified alternatives are widely utilized in animal feed industry. Consequently, these kinds of situations are likely to decrease productivity of animal farms and increase cost of using alternatives toward antibiotics subsequently it would increase animal production cost.

Diverse studies have conducted to resolve these problematic situations. As a part of this, the investigation of enzyme is researched as feed additives. Amongst the biotechnological additives, feed enzymes are seen perhaps as having made the most progress and impact in the past decade, following the extensive and increasing use of crystalline amino acids, many of which are also produced from industrial fermentation. Bonneau and Laarveld (1999) identified the use of enzyme in a number of applications for improving feed efficiency in animals. Enzyme supplementation in weaning pigs' diet is regarded very

important issue because of the levels of dietary fiber in weaning pig's diet. Dietary fiber in animal feed is considered as an 'anti-nutritional factor', due to their negative influence on digestion and absorption of nutrients such as starch and protein in the gut of monogastric animals, especially in weaning pigs. High level of dietary fiber resulted in increasing the occurrence of diarrhea in weaning pigs subsequently it might influence negatively on growth performance of young animals

β-mannanase is a kind of carbohydrase, an enzyme for digesting a mannan component which breaks down hemicellulose to MOS or mannose. Especially in the case of MOS, it prevented colonization of pathogenic microorganism, increased activity of macrophages and T-lymphocytes (Walsh et al., 1993). Consequently, it enhanced growth performance of weaning pigs as well as nutrient digestibility.

Hemicell-HT® is a enzyme which break down β-1,4-mannan linkage, made of fermented *bacillus lentus*. Many studies reported that supplementation of Hemicell-HT® not only increased mannan efficiency which contains in soybean meal, but also decreased frequency of diarrhea. However, adequate supplementation level is not clearly defined. Therefore, this study was conducted to investigate optimum levels of Hemicell-HT® supplementation to weaning pigs' diets on growth performance, nutrient digestibility, blood analysis and intestinal integrity.

Materials and Methods

Experimental animals and feeding

A total of 96 crossbred pigs ([Landrace × Yorkshire]) × Duroc) with an average body weight of 6.96 ± 0.70kg were used for 5weeks feeding trial in Seoul National University experimental farm located in Suwon city, Kyungki-Do. Pigs were allotted into 4 treatments with 6 replicates and 4 pigs per pen in a randomized complete block (RCB) design. Pigs were housed in environmentally controlled weaning pig's pen which was easy to supply feed and water *ad libitum* and control room temperature. Body weight and the feed intake were recorded at 2 and 5 weeks to calculate average daily gain (ADG), average daily feed intake (ADFI) and Gain/Feed ratio of the pigs

Experimental design and diet

The treatments included 1) NC (negative control); (basal diet), 2) PC (positive control); (basal diet + 0.1 % antibiotics), 3) A (basal diet + 0.04 % Hemicell-HT®), 4) B (basal diet + 0.06 % Hemicell-HT®). This trial was conducted with corn-soybean meal based diet and two phase feeding programs were used. Phase I diet, contained 23.7 and 1.35% of crude protein and lysine, respectively, was supplied for the first 2 weeks. Phase II diet, contained 20.9 and 1.15% of crude protein and lysine, respectively, was provided to pigs for the last 3 weeks. All other nutrients were met or exceeded nutrient requirements of NRC (1998). The formula and chemical composition of basal diet in all phases are presented in Tables 1 and 2.

Blood assay

Blood was collected from their anterior vena cava at initial, 2 and 5 weeks from randomly selected six pigs in each treatment. The glucose, triglycerides, lactic acid and APP level were analyzed with these blood samples. These collected samples were centrifuged for 15 min by 3,000 rpm at 4℃. Then, pure sera samples were aspirated by pipette and stored at -20℃ until analyzed.

Digestibility trial

A total of 20 barrow piglets were additionally allocated to 4 treatments with 4 replicates and housed in an individual metabolic cage fitted with urine and feces separators in a completely randomized design (CBD). Initial body weight of piglets had approximately 10.17 ± 1.35 kg. The cages were located in an environmentally controlled room, maintained at a constant temperature of 27℃ (±2℃). The trial consisted of an initial 7 days adaptation period subsequently feed intake and feces output were recorded for further 5 days. The experimental diets were supplied daily twice at 2.0% of body weight ration. The total amounts of feed consumed and excreta were dried in a air forced drying oven at 60℃ for 72 hour and ground to 1 mm in a Wiley mill for chemical analysis.

Anatomy trial

A total of 20 pigs were housed in a plastic woven floored pen, equipped with a feeder and a nipple waterer and allowed *ad libitum* access to feed and water throughout the whole experimental period. Five pigs were selected from each treatment at the 21st day of the experiment. The selected pigs were moved to individual cage and fasted overnight. Feed was supplied twice at 0 and 4 hr before anatomy, the pigs were slaughtered after 40 minute after last feeding and small intestine was collected. About 2 cm segments from the small intestinal midpoint were removed, rinsed with 0.4 M KCl (Cera et al., 1988) to remove foreign materials. Immediately, two 2 mm^2 tissue samples were cut from this section and submerged in a primary fixing solution (0.05M sodium carbohydrate buffer, pH 7.2) containing 2% paraformaldehyde and 2% glutaraldehyde and stored at 4℃. Then, all of the contents from each organ were mixed and analyzed. To measure intestinal morphology for villus height and crypt depth, after 1 or 2 days fixation in 10% neutral buffered formalin, midpoint of intestinal tract were dehydrated through graded alcohols and a xylene step and embedded in a hematoxylin and eosin (HE)-stained sections (4㎛).

Moisture contents of feces

Pure fecal samples were collected from 5 piglets in each treatment within 24 hours by rectal dilatation. The AOAC (1990) procedure 967.03 was applied to measure moisture contents of feces, taken 1 g of sample per treatment and dried in 3 hours at 105℃ drying oven. After drying, samples were discharged in desiccators for 30 min.

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

Statistical analysis

Analysis of experimental diets and excreta was conducted according to the methods of the AOAC(1995). Statistical analysis was performed using the General Linear Model (GLM) procedure of SAS (2004). Pen was the experimental unit for analysis of performance data. For nutrient digestibility and immunological parameters, analyses were performed using the GLM procedure of SAS with individual pig as the experimental unit by comparing means according to the least significantly difference (LSD). Differences were considered significant at the level of $P<0.05$ and highly significant at the level of $P<0.01$.

Results and Discussion

Growth performance

Table 3. presented the effects of supplementation of Hemicell-HT® on growth performance in weaning pigs. During phase I (0~2 week), antibiotics supplemented treatment (PC) showed no difference on body weight with non-antibiotic treatment ($P>0.05$) as well as average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F ratio). However, during phase II (2~5 week), PC was significantly higher among all treatments on BW ($P=0.001$) and ADG ($P=0.001$). During the total 5 weeks experimental period, PC and pigs fed 0.06% of Hemicell-HT® showed significant improvement on BW ($P=0.001$) and ADG ($P=0.014$). This result demonstrated that supplementation of Hemicell-HT® treatments showed improved growth performance in weaning pigs compared to NC treatment although antibiotics treatment was the highest performance among treatments.

In Phase II, however, improved ADG and G:F ratio was observed when weaning pigs were fed 0.06% Hemicell-HT® treatment diet. In Phase I, improved performance was not observed when pigs were fed diets with β-mannanase. This result represented that digestive organs of weaning pigs' were not developed completely yet until 2 weeks postweaning but positive response was observed by supplementation of Hemicell-HT® after 2 weeks due to the fact that gastrointestinal tracts and digestive organs were developed subsequently digestive enzymes were secreted successfully except undigestible nutrients such as mannan or fiber fraction. In this time, a positive

response was observed by supplementation Hemicell-HT® because mannan was a kind of undigestible fraction of weaning pigs' diet.

Although mannan content of dehulled soybean meal is relatively low (1.3%; ChemGen, unpublished data), the addition of β-mannanase to the diet appeared to improve growth efficiency of weaning pigs in late weaning phase. The increased efficiency observed in the present study could potentially indicate an energy advantage by supplementation of exogenous enzyme to corn-SBM based diet. In fact, recent work (D. Fodge, unpublished data) has demonstrated that pigs fed a diet with decreased digestible energy (−100 kcal/kg) plus β-mannanase addition in the diet had similar ADG and G:F ratio compared with pigs fed a high erenergy diet with no enzyme supplementation. These results suggested that β-mannanase may provide additional digestible energy (100 kcal/kg) of DE to a typical swine diet because undigestible fractions can be digested with the help of β-mannanase.

Pettey et al. (2002) reported that a β-mannanase preparation added to corn-soybean meal-based diets improved feed efficiency in late-nursery pigs. Kim et al. (2003) demonstrated that supplementation of carbohydrase composed of α-1,6-galactosidase and β-1,4-mannanase can increase growth performance in nursery pigs by improving the digestibility of energy and amino acids in corn-soybean meal-based diet.

These studies supported the idea that supplementation of β -mannanase in weaning pig's diet is an efficient, practical method of reducing the NSPs and anti-nutritional effects of soybeans (Sugimoto and Van Buren, 1970). Moreover, as an alternative agent to antibiotics, addition of Hemicell-HT® was not found similar effect for the piglets'

growth performance compared with antibiotics supplementation. However, 0.06% of Hemicell-HT® supplementation may have a potential alternative material to antibiotics to improve growth performance.

Nutrient digestibility

Table 4. presented the effect of Hemicell-HT® on nutrient digestibility and nitrogen retention at phase Ⅱ. Except for crude fat digestibility, the statistical differences were observed on neither nutrient digestibility nor N retention ($P<0.01$). When pigs were fed 0.06% of Hemicell-HT® treatment diet, significantly higher crude fat digestibility, was observed among Hemicell-HT® treatment diets. Negative control group and supplementation of 0.04% Hemicell- HT® treatment showed the lowest urinary N excretion, resulting in the lowest N retention.

Results of previous studies about effect of β-mannanase supplementation on nutrient digestibility are prone to inconsistent. Dale (1997) demonstrated that mannan decreased nitrogen retention and fat absorption due to its high viscosity. Radcliffe et al. (2001) reported the addition of β-mannanase to corn-soybean meal based diets led to an increased apparent ileal digestibility (AID) of dry matter and apparent total track digestibility (ATTD) of energy. However, Petty et al. (2002) reported that the addition of β-mannanase had no significant improvement on nitrogen retention.

In the present experiment, crude fat digestibility improved significantly ($P<0.05$) by β-mannanase supplementation although N retention did not show significant difference among treatments.

The morphological effects

The effects of Hemicell-HT® on intestinal growth and morphology in weaning pigs is presented in Table 5. The scanning electron microscopy (SEM) revealed intestinal morphology in small intestinal section obtained among treatments at 3 week of post weaning. Normal types of villi, a long and finger-shaped, were found in sucking period. Cera et al. (1988) reported that the villi height was dramatically declined within 3 d of weaning, and it was increased slowly. Supplementation of Hemicell-HT® had no effect on villi height and crypt depth. When pigs were fed 0.06% of Hemicell-HT® treatment diet, similar villus height in jejunum and V:C ratio in jejunum and ileum are observed. However, in the ratio of villi height and crypt depth was not improved by supplementation of antibiotics.

The changes in gut morphology nutrient digestibility would directly effect on growth performance of weaning pigs. Numerous studies have demonstrated that small intestine of the early-weaned pig undergoes major changes in villus morphology and reductions in specific enzyme activity during the immediate post-weaning period (Armstrong and Clawson, 1980; Hampson and Kidder, 1986; Pluske et al., 1995; Beers-Schreurs et al., 1998). Pluske et al. (1995) reported that reduction of villus height and increase in crypt depth in the small intestine after weaning are generally associated with reductions in the specific activity of the lactase and sucrase. Cera et al. (1988) observed that shortening of the microvilli during post weaning would also reduce total luminal villous absorptive area.

Blood profile

Table 6. showed the effects of Hemicell-HT® on blood profile. Glucose is another important oxidative fuel for the gut, although less significant quantitatively than glutamate, glutamine, and aspartate. Glucose is often considered as a "primal" oxidative fuel for most mammalian tissues (Girard et al., 1992) A majority of the glucose carbon utilized by gut tissues is either metabolized to lactate and alanine or used for biosynthetic purposes (Ellis et al., 1996). In vivo studies with pigs demonstrated that of the relatively small fraction of dietary glucose utilized by the PDV tissues, 45% and 37% is metabolized to lactate and alanine, respectively (Stoll et al., 1999). This implied that a large proportion of intestinal glucose requirement, which is derived largely from the arterial circulation, maybe used for biosynthesis of structural and functional molecules, such as mucin glycoproteins, fatty acids and lipids.

Prebiotics such as fructo-oligosaccharides have been regarded to stimulate the growth of *lactobacilli* and bifidobacteria in the intestine, but there was no evidence that these probiotics influence gut integrity in weaning pigs. *Lactobacilli* produce lactic acid, which is known to have antibacterial activity. It meant lactic acid concentration in the blood could be used as health parameter of small intestine. Presumably, when pigs were fed 0.06 % Hemicell-HT® treatment diet, lactate utilization might be affected positively by dietary enzyme. It could be explained that Hemicell-HT® functioned as prebiotics so that it made beneficial environment for production of *Lactobacilli* in the small intestine.

Generally it is very well known that fats and oils in the diet are digested prior to absorption by the gut. Complete hydrolysis of all three fatty acid groups from the glycerol backbone occurs in the gut. Triglyceride (TG) metabolism is regulated at the points of TG hydrolysis at the cell, fatty acid synthesis, and fatty acid transport into the mitochondrion, as well as other points (Kiran et al., 2000)

The acute phase proteins (APP) are a group of blood proteins that contribute to restoring homeostasis and limiting microbial growth in an antibody-independent manner in animals subjected to infection, inflammation, surgical trauma or stress. In the last two decades, many advances have been made in monitoring APP in both farm and companion animals for clinical and experimental purposes. Also, the mechanism of the APP response is receiving attention in animal science in connection with the innate immune systems of animals. Although we found no significant differences among treatments, Hemicell-HT® treatments showed lower APP level compare to any other treatments. Especially, addition of 0.06% Hemicell-HT® in the weaning pigs diet are positively influenced on reducing APP level which meant effectively acted as degrading innate stress factors of weaning pigs.

Moisture contents of feces

Table 7. showed the effect of each treatment on moisture contents of feces. Positive control had lower moisture contents compared to negative control in phase Ⅱ ($P<0.05$). The pigs fed diets supplemented of 0.04% Hemicell-HT® showed similar moisture contents of feces compared to negative control in all phases, and had

statistically equal effect on moisture contents of feces as positive control ($P<0.05$).

Bayley (1970) and Carlson (1970), while studying digestive problems in baby pigs, determined that the total amount of undigested starch in the lower digestive tract was higher in pigs consuming a complex cereal-based diet as compared with a semipurified diet. Normal fecal material was a semisolid containing up to approximately 70% moisture. Percentage moisture could possibly be used as an index to severity of diarrhea. Soluble fiber components may cause a thickness of the components of the pig's gut. Generally, it is known that soluble NSP increased intestinal transit time, delayed gastric emptying and glucose absorption, increased pancreatic secretion and slowed absorption, whereas insoluble NSP decreased transit time, enhanced water-holding capacity and assisted in fecal bulking (Low, 1985). In this experiment, same amount of barley was used as soluble fiber in each treatment, but it might cause increasing fecal viscosity if higher level of barley was added in the diet. It might be explained that Hemicell-HT® was affected on diarrhea occurrence by degrading NSPs in the barley and soybean meal, consequently converted into energy sources efficiently.

Conclusion

The purpose of this study was to evaluate the effects of Hemicell-HT® supplementation in weaning pigs' diet. In this experiment, growth performance, blood profiles, nutrient digestibility, the

morphological effect, blood profile and moisture contents of feces were measured. In growth, 0.06% Hemicell-HT® treatment showed significantly higher performance among non-antibiotics treatments. No significant differences were observed in the glucose, lactate, APP level, triglycerides among all treatments. In nutrient digestibility, the pigs fed 0.06% Hemicell-HT® treatment diet showed higher crude fat digestibility. The ratio of villi height and crypt depth in gastrointestinal tract was higher when pigs were fed 0.06% of Hemicell-HT® treatment diet. In moisture contents of feces, supplementation of 0.04% Hemicell-HT® showed similar score compared with negative control in all phases, and had statistically equal effect as positive control ($P < 0.05$). From this study, addition of Hemicell-HT® to weaning pig's diet can be effective alternative feed supplement instead of antibiotics based upon growth performance of weaning pig. Moreover, it influences positively on small intestine integrity of weaning pig, relatively improved on mortality and moisture contents of feces. It could be explained that these β-mannanases catalyze degrading mannans efficiently, acting as mannan-oligosaccharides (MOS). Considering enzyme level in weaning pigs' diet, supplementation 0.06% of Hemicell-HT® is recommended based upon the present study.

Table 1. Formula and chemical composition of phase Ⅰ.

	NC	PC	A	B
Ingredients, %				
Corn	17.77	17.56	17.73	17.63
SBM 44% CP	32.73	32.80	32.80	32.71
HP300[1]	7.69	7.68	7.66	7.73
Whey powder	3.18	3.14	3.10	3.19
Lactose	12.00	12.00	12.00	12.00
Barley	24.00	24.00	24.00	24.00
Soy-oil	0.00	0.09	0.04	0.05
MCP	1.01	1.01	1.01	1.01
Limestone	0.92	0.92	0.92	0.92
L-Lysine·HCl	0.13	0.13	0.13	0.13
Vitamin Mix[2]	0.12	0.12	0.12	0.12
Mineral Mix[3]	0.12	0.12	0.12	0.12
Salt	0.10	0.10	0.10	0.10
Choline-Cl(25%)	0.10	0.10	0.10	0.10
ZnO	0.10	0.10	0.10	0.10
Hemicell-HT®	0.00	0.00	0.04	0.06
Avilamycin	0.00	0.05	0.00	0.00
Tyrosin	0.00	0.05	0.00	0.00
Total	100.00	100.00	100.00	100.00
Chemical composition				
Total ME, kcal/kg	3265.00	3265.00	3265.00	3265.00
Total crude protein, %	23.70	23.70	23.70	23.70
Total lysine, %	1.35	1.35	1.35	1.35
Total methionine, %	0.35	0.35	0.35	0.35
Total Ca, %	0.80	0.80	0.80	0.80
Total P, %	0.65	0.65	0.65	0.65

[1] HP300 (Hamlet protein, Horsens, Denmark).

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

[3] Provided the following quantities of minerals per kg of complete diet: Se, 0.1 mg; I,0.3 mg; Mn, 24.8 mg; Cu·SO_4, 54.1mg; Fe, 127.3 mg; Zn, 84.7 mg; Co, 0.3 mg.

Table 2. Formula and chemical composition of phase Ⅱ.

	NC	PC	A	B
Ingredients, %				
Corn	36.21	36.02	36.13	36.09
SBM 44% CP	29.10	29.00	29.04	29.07
HP300[1]	3.72	3.83	3.79	3.76
Lactose	4.00	4.00	4.00	4.00
Barley	24.00	24.00	24.00	24.00
Soy-oil	0.69	0.77	0.72	0.74
MCP	0.90	0.90	0.90	0.90
Limestone	0.70	0.70	0.70	0.70
L-Lysine·HCl	0.14	0.14	0.14	0.14
Vitamin Mix[2]	0.12	0.12	0.12	0.12
Mineral Mix[3]	0.12	0.12	0.12	0.12
Salt	0.10	0.10	0.10	0.10
Choline-Cl(25%)	0.10	0.10	0.10	0.10
ZnO	0.10	0.10	0.10	0.10
Hemicell-HT®	0.00	0.00	0.04	0.06
Avilamycin	0.00	0.05	0.00	0.00
Tyrosin	0.00	0.05	0.00	0.00
Total	100.00	100.00	100.00	100.00
Chemical composition				
Total ME, kcal/kg	3,265.00	3,265.00	3,265.00	3,265.00
Total crude protein, %	20.9	20.9	20.9	20.9
Total lysine, %	1.15	1.15	1.15	1.15
Total methionine, %	0.30	0.30	0.30	0.30
Total Ca, %	0.70	0.70	0.70	0.70
Total P, %	0.60	0.60	0.60	0.60

[1] HP300 (Hamlet protein, Horsens, Denmark).

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

[3] Provided the following quantities of minerals per kg of complete diet: Se, 0.1 mg; I,0.3 mg; Mn, 24.8 mg; Cu·SO_4, 54.1mg; Fe, 127.3 mg; Zn, 84.7 mg; Co, 0.3 mg.

Table 3. Effects of Hemicell-HT® supplementation on growth performance in weaning pigs [1,2]

Criteria	Treatments				SEM[4]	P-value
	NC [3]	PC	A	B		
Body weight (kg)						
Initial	6.96	6.97	6.98	6.95	0.20	0.62
2 week	9.18	9.56	9.08	9.37	0.25	0.27
5 week	16.61^{b}	18.44^{a}	15.86^{b}	17.01^{ab}	0.49	0.01
ADG (g)						
0-2 week	158	185	150	173	6.06	0.25
3-5 week	354^{b}	423^{a}	323^{b}	364^{b}	14.06	0.01
0-5 week	276^{b}	328^{a}	254^{b}	288^{ab}	10.40	0.01
ADFI (g)						
0-2 week	283	303	281	295	7.99	0.43
3-5 week	742	809	728	836	22.31	0.30
0-5 week	558	607	549	537	15.12	0.23
G:F ratio						
0-2 week	0.557	0.611	0.534	0.585	0.01	0.15
3-5 week	0.481	0.522	0.442	0.445	0.01	0.09
0-5 week	0.491	0.540	0.461	0.536	0.01	0.10

[1] Least squares mean for six pens/ treatment with four pigs/ pen.

[2] A total 96 crossbred pigs was fed from average initial body 6.96 ± 0.70 kg and the average final body weight was 16.82 kg.

[3] Phase I, II - NC: Basal diet, PC: Basal diet + 0.1% antibiotics(avilamycin 0.05% + tyrosin 0.05%), A: Basal diet + Hemicell-HT® 0.04%, B: Basal diet + Hemicell-HT® 0.06%.

[4] Standard error of the means.

[a,b] Means with different superscripts in the same row significantly differ ($P<0.01$).

Table 4. Effects of Hemicell-HT® supplementation on nutrient digestibility and nitrogen retention in weaning pigs[1]

Criteria	Treatments				SEM[3]	P-value
	NC[2]	PC	A	B		
Nutrient digestibility (%)						
Dry matter	87.92	87.44	87.95	87.78	0.34	0.85
Crude protein	86.63	86.44	87.82	86.56	0.48	0.91
Crude ash	56.14	54.14	53.27	58.30	1.34	0.73
Crude fat	53.48[c]	70.35[ab]	68.23[b]	80.51[a]	2.64	0.01
Nitrogen retention (%)						
N intake	6.79	6.58	7.03	7.05	—	—
Fecal N	0.91	0.89	0.86	0.95	0.03	0.96
Urinary N	1.77	2.17	2.47	1.86	0.10	0.25
N retention[4]	4.11	3.51	3.70	4.24	0.12	0.13

[1] Least squares means four pigs/ treatment in an individual pen.

[2] Phase I, II - NC: Basal diet, PC: Basal diet + 0.1% antibiotics (avilamycin 0.05% + tyrosin 0.05%), A: Basal diet + Hemicell-HT® 0.04%, B: Basal diet + Hemicell-HT® 0.06%.

[3] Standard error of the means.

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

Table 5. Effects of Hemicell-HT® supplementation on intestinal morphology in weaning pigs[1,2]

Criteria	Treatment				SEM[4]	P-value
	NC [3]	PC	A	B		
Villus height and crypt depth in jejunum						
Villus height (㎛)	366.92	369.92	372.67	428.75	13.60	0.32
Crypt depth (㎛)	231.42	244.67	272.33	212.5	10.51	0.39
V:C ratio	1.62	1.57	1.45	2.04	0.08	0.07
Villus height and crypt depth in ileum						
Villus height (㎛)	340.42	309.67	299.25	378.08	13.79	0.22
Crypt depth (㎛)	188.83	175.67	191.92	191.58	8.21	0.87
V:C ratio	1.83	1.75	1.66	2.08	0.10	0.91

[1] Least squares means for five pigs/ treatment.
[2] All pigs, average BW 13.37 ± 2.13 kg, were anatomized at d 21 of experiment.
[3] Phase I, II - NC: Basal diet, PC: Basal diet + 0.1% antibiotics (avilamycin 0.05% + tyrosin 0.05%), A: Basal diet + Hemicell-HT® 0.04%, B: Basal diet + Hemicell-HT® 0.06%.
[4] Standard error of the means.

Table 6. Effects of Hemicell-HT® supplementation on blood profile in weaning pigs[1]

Criteria	Treatments				SEM[3]	P-value
	NC[2]	PC	A	B		
Triglycerides(mg/dL)						
Initial	34.33	34.33	34.33	34.33	-	-
5 week	34.60	30.60	34.60	53.00	2.86	0.18
Lactic acid(mg/dL)						
Initial	61.00	61.00	61.00	61.00	-	-
5 week	59.30	51.30	54.90	72.00	4.14	0.15
Glucose(mg/dL)						
Initial	128.00	128.00	128.00	128.00	-	-
5 week	96.60	100.80	99.80	103.40	2.29	0.85
APP level(ng/L)						
Initial	1,800	1,800	1,800	1,800	-	-
5 week	625	650	550	350	95.50	0.53

[1] Least squares means for five pigs/ treatment.

[2] Phase I, II - NC: Basal diet, PC: Basal diet + 0.1% antibiotics (avilamycin 0.05% + tyrosin 0.05%), A: Basal diet + Hemicell-HT® 0.04%, B: Basal diet + Hemicell-HT® 0.06%.

[3] Standard error of the means.

Table 7. Effects of Hemicell-HT® supplementation on moisture content of feces in weaning pigs[1]

Criteria	Treatments				SEM[3]	P-value
	NC[2]	PC	A	B		
Moisture content of feces (%)						
2 week	74.25	73.10	74.36	78.74	0.86	0.12
5 week	79.84[a]	74.16[b]	76.33[ab]	77.72[a]	0.60	0.02

[1] Least squares means for five pigs/ treatment.

[2] Phase I, II - NC: Basal diet, PC: Basal diet + 0.1% antibiotics(avilamycin 0.05% + tyrosin 0.05%), A: Basal diet + Hemicell-HT® 0.04%, B: Basal diet + Hemicell-HT® 0.06%.

[3] Standard error of the means.

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

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Ⅴ. Summary in Korean

본 실험은 mannanase의 일종인 Hemicell-HT® 가 이유 자돈의 성장 능력과 혈액 성상 그리고 소장의 형태에 미치는 영향에 대해 조사하였다. 총 96두의 28일령 이유자돈 ([Yorkshire × Landrace]×Duroc)을 5처리 6반복 돈방당 4두씩 공시하였다. 실험돈은 체중과 성별에 따라 난괴법 (randomized complete block (RCB) design)으로 배치하였다. 처리구는 phase Ⅰ(0-2주), Ⅱ(3-5주)에서 1) NC (항생제 무첨가 기초사료), 2) PC (기초사료 + 항생제 0.10% 첨가), 3) A (기초사료 + Hemicell-HT® 0.04%), 4) B (기초사료 + Hemicell-HT® 0.06%)로 구성되었다. 총 5주간의 자돈기 성장 능력에서는 항생제를 첨가한 PC 처리구와 0.06% Hemicell-HT® 처리구가 유의적으로 높은 체중과 일당증체량을 나타내었다(P<0.01). 영양소소화율에서는 0.06%의 Hemicell-HT® 을 첨가한 처리구의 조지방 소화율이 유의적으로 높은 수치를 나타내었다(P<0.01). 폐사율에 있어 사양실험 기간 동안 폐사한 개체가 없었으며, 혈액 분석을 통한 처리구별 glucose, lactic acid, triglycerides 함량에서는 유의차가 나타나지 않았다. 분변의 점도에 있어서는 항생제 처리구와 Hemicell-HT® 0.06%첨가 처리구가 동등한 분변 점도를 나타내었다(P<0.05). 자돈의 융모와 융와의 비율을 보았을 때 PC 처리구에 비해 항생제 무첨가구에서 개선되는 경향을 보였으며(P=0.10), 수치상으로 사료 내 0.06%의 Hemicell-HT® 을 첨가한 처리구의 V : C ratio 가 가장 높게 나타나, 소장의 건강성에 긍정적인 작용을 하는 것으로 나타났다. 결과적으로 Hemicell-HT® 0.06%의 첨가가 자돈의 성장성적과 소장의 건강성 측면에서 긍정적인 효과를 보이는 것으로 생각된다.

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