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A Dissertation
for the Degree of Doctor of Philosophy

**Impacts of β -glucan with Vitamin E
Supplementation in Weaner, Grower/Finisher,
and Lactating Sow**

이유자돈, 육성비육돈, 포유돈 사료 내
베타글루칸과 비타민 E 첨가가 미치는 영향

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Overall Summary

Impacts of β -glucan with Vitamin E Supplementation in Weaner, Grower/Finisher, and Lactating Sow

Antibiotics have been widely used in the feed for a long time because supplementing antibiotics in the diet usually improved growth performance and feed efficiency in swine (Cromwell, 2002). However, the use of antibiotics in swine feed has been forbidden due to concerns about the development of antibiotic resistance and human health risks of antibiotic residues. Therefore, many antibiotic alternatives have been studied by researchers such as probiotics, prebiotics, enzymes, plant extracts, organic acids, and medium chain triglycerides to maintain pig health and performance (Dierick et al., 2002).

Among these alternatives, β -glucan which is present in cellulose in plants, bran of cereal grains, cell walls of baker's yeast, certain fungi, mushrooms, and bacteria activates the immune system by binding to receptors on the surface of innate immune cells (Vetvicka et al., 2014). It also has a beneficial effect on pig growth because it induces a specific immune response and increases non-specific immunity and resistance to oral antigens as an immune-modulator (Hahn et al., 2006)

Vitamin E which is a fat-soluble vitamin and is a generic term for all tocol and tocoterienol derivatives that exhibit varying degrees of biological activity plays important roles in antioxidation, immune system, and growth performance (Combs, 1998; Pinelli-Saavedra, 2003)

There are several previous experiments about β -glucan and vitamin E respectively. However, there are almost none experiment about supplementing β -glucan and vitamin E together. Three experiments were conducted to investigate the impacts of β -glucan with vitamin E supplementation in weaning pigs (Experiment 1), in growing and finishing pigs (Experiment 2), and in lactating sows (Experiment 3).

Experiment I. Effects of β -glucan with Vitamin E Supplementation on the Growth Performance, Blood Profiles, Immune Response, Fecal Microbiota, Fecal Score, and Nutrient Digestibility in Weaning Pigs

This study was conducted to evaluate effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, fecal microbiota, fecal score, and nutrient digestibility in weaning pigs. A total of 200 weaning pigs with an average body weight (BW) of 7.64 ± 0.741 kg were allotted to five treatment groups and were divided based on sex and initial BW in four replicates with ten pigs per pen in a randomized complete block design. The experimental diets included a corn–soybean meal-based basal diet with or without 0.1% or 0.2% β -glucan and 0.02% vitamin E. The pigs were fed the diets for 6 weeks. A total of 15 barrows were used to evaluate the nutrient digestibility by the total collection method. The BW and feed intake were measured at the end of each phase. Blood samples were collected at the end of each phase, and fecal samples were collected at the end of the experiment. The addition of β -glucan with vitamin E to weaning pig feed increased BW, average daily gain, and average daily feed intake. A significant decrease in yeast & mold and Proteobacteria and a tendency for Lactobacillus to increase compared to the control was shown when 0.1% β -glucan with 0.02% vitamin E were added. The fecal score in weaning pigs was lower in the treatments supplemented with 0.1% or 0.2% β -glucan and 0.02% vitamin E compared to the control. In addition, vitamin E was better supplied to weaning pigs by increasing the concentration of α -tocopherol in the blood of weaning pigs when 0.02% vitamin E was supplemented. However, there was no significant difference not only in the immune response but also in the nutrient digestibility. Consequently, 0.1% β -glucan with 0.02% vitamin E in a weaning pig's diet were beneficial to the growth performance of weaning pigs by improving intestinal microbiota and reducing the incidence of diarrhea.

Experiment II. Effects of β -glucan with Vitamin E Supplementation on the Growth Performance, Blood profiles, Immune Response, Pork Quality, Pork Flavor, and Economic Benefit in Growing and Finishing Pigs

This study was conducted to evaluate the effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, pork quality, pork flavor, and economic benefit in growing and finishing pigs. A total of 140 growing pigs ([Yorkshire x Landrace]) x Duroc) were assigned to five treatments considering sex and initial body weight (BW) in 4 replications with 7 pigs per pen in a randomized complete block design. The experimental diets included a corn-soybean meal based basal diet with or without 0.05% or 0.1% β -glucan and 0.02% vitamin E. The pigs were fed the diets for 12 weeks (phase I : 0-3, phase II : 3-6, phase III : 6-9, phase IV : 9-12). The BW and feed intake were measured at the end of each phase. Blood samples were collected at the end of each phase. Four pigs from each treatment were selected and slaughtered for meat quality. Economic benefit was calculated considering the total feed intake and feed price. Pork flavor was analyzed through inosine monophosphate analysis. The average daily gain and feed efficiency were improved compared to the control when β -glucan or vitamin E was added. Supplementing 0.05% β -glucan significantly increased lymphocyte concentration compared to the addition of 0.1% β -glucan and the content of vitamin E in the blood increased when 0.02% vitamin E was added. The HBE treatment with 0.1% β -glucan and 0.02% vitamin E showed the most economic effect because it had the shortest days to market weight and the lowest total feed cost. The addition of β -glucan or vitamin E had a positive role in improving the flavor of pork when considering that the content of inosine monophosphate was increased. However, carcass traits and meat quality were not affected by β -glucan or vitamin E. Consequently, the addition of 0.1% β -glucan and 0.02% vitamin E in growing and finishing pig's diet showed great growth performance and economic effects by supplying vitamin E efficiently and by improving the health condition of pigs due to β -glucan.

Experiment III. Effects of β -glucan with Vitamin E Supplementation on the Physiological Response, Litter Performance, Blood Profiles, Immune Response, and Milk Composition of Lactating Sows

This study was conducted to evaluate the effects of β -glucan with vitamin E supplementation on the physiological response, litter performance, blood profiles, immune response, and milk composition of lactating sows. A total of 50 multiparous F₁ sows (Yorkshire \times Landrace) with an average body weight (BW) of 233.6 ± 4.30 kg and an average parity of 4.00 ± 0.307 and their litters were used in this experiment. All sows were allotted to one of five treatments, taking into consideration BW, backfat thickness, and parity in a completely randomized design with 10 replicates. The experimental diets included a corn–soybean meal-based basal diet with or without 0.1% or 0.2% β -glucan and 110 IU vitamin E/kg diet. All treatments added with β -glucan or vitamin were statistically higher in the average daily feed intake (ADFI) of lactating sows compared to those of the control (Diet, $p < 0.01$). Additionally, the ADFI of lactating sows was significantly higher in the groups supplemented with 0.1% β -glucan compared to 0.2% β -glucan (BG, $p < 0.01$). There was an increasing trend in piglet weight at weaning (BG, $p = 0.07$), litter weight at the 21st day of lactation (BG, $p = 0.07$) and litter weight gain (BG, $p = 0.08$) in groups supplemented with 0.1% β -glucan. The addition of 110 IU vitamin E/kg diet increased vitamin E concentration significantly in lactating sows (VE, $p < 0.01$) and exhibited a trend for higher concentrations of vitamin E (VE, $p = 0.09$) in piglets. Adding 0.1% β -glucan compared to 0.2% β -glucan showed a lower trend in TNF- α concentration in lactating sows (BG, $p = 0.06$) and in piglets (BG, $p = 0.09$) on the 21st day of lactation. There were no significant differences in the milk composition of sows. Consequently, adding 0.1% β -glucan and 110 IU vitamin E/kg to a lactating sow's diet was beneficial to the growth performance of piglets by leading to an increase in the feed intake of sows and efficiently supplying vitamin E to both the sows and piglets.

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List of Abbreviation

ADG	:	Average daily gain
ADFI	:	Average daily feed intake
APC	:	Aerobic plate count
ANOVA	:	Analysis of variation
ATP	:	Adenosine triphosphate
BW	:	Body weight
CP	:	Crude protein
DFM	:	Direct-fed microbials
DM	:	Dry matter
FCR	:	Feed conversion ratio
G:F ratio	:	Gain to feed ratio
IL	:	Interleukin
IMP	:	Inosine monophosphate
IU	:	International unit
MCFA	:	Medium-chain fatty acids
MDCP	:	Mono-dicalcium phosphate
ME	:	Metabolizable energy
NRC	:	National research council
SAS	:	Statistical analysis system
SBM	:	Soybean meal
SCFA	:	Short chain fatty acid
SEM	:	Standard error of the mean
SNF	:	Solid not fat
TNF- α	:	Tumor necrosis factor- α
WEI	:	Weaning to estrus interval
WHC	:	Water holding capacity

Chapter I. General Introduction

Antibiotic in swine feed have been widely supplemented for a long time to increase growth performance, feed utilization and to prevent disease in livestock feeding environments (Cromwell, 2002). However, the use of antibiotics in animal feed has been banned in many countries including the European Union since 2006 and Korea since 2011 because of bacterial resistance to antibiotics and the dangers of antibiotic residues to human health (Nguyen et al., 2018). Therefore, there is increasing interest in alternatives such as probiotics, prebiotics, enzymes, plant extracts, organic acids, and medium chain triglycerides to maintain pig health and performance (Dierick et al., 2002).

Among these alternatives, β -glucans, polysaccharides or dietary fibers composed of D-glucose monomers linked by β -glycosidic bonds, are naturally found in cellulose in plants, bran of cereal grains, cell walls of baker's yeast, certain fungi, mushrooms, and bacteria (Park et al., 2018). β -glucans are immunomodulator that can enhance the host immune function. They activate macrophages, neutrophils and increase plasma leucocytes counts and lymphocyte proliferation activity (Metzler-Zebeli et al. 2011; Zhou et al. 2013). In addition, β -glucans could maintain blood cholesterol concentrations (Vetvicka et al., 2014). They also have beneficial effects on pig growth performance because they induce specific immune response and increase non-specific immunity and resistance to oral antigens as an immunomodulator. There are many research about supplementing β -glucans could help growth performance in weaning pig periods (Schoenherr et al., 1994; Hahn et al., 2006; Zhou et al., 2013; Wu et al., 2018). In addition, Luo et al. (2014) reported that β -glucans supplementation increased the body weight gain and improved FCR in growing and finishing pig periods. Also, the ADFI increased linearly from 0% to 0.02% β -glucan treatments in lactating sows (Szuba-Trznadel et al., 2014).

Other immunomodulators include antioxidant molecules, such as vitamin E. Vitamin E which is a fat-soluble vitamin and is a generic term for all tocol and tocoterienol derivatives that exhibit varying degrees of biological activity plays

important roles in antioxidation, immune system, and growth performance (Combs, 1998; Pinelli-Saavedra, 2003). Vitamin E is the major antioxidant that breaks chains in the body tissues and is considered the first line of defense against lipid peroxidation, protecting cell membranes in the early stages of free radical attack through its free radical scavenging activity (Pinelli-Saavedra, 2003). Also, supplementation of vitamin E on diets has a considerable potential effect in increasing resistance in the sow and the neonatal pig to enteric diseases such as *E.coli* (Pharazyn et al., 1990).

There are several previous experiments about β -glucan and vitamin E, respectively. However, there are almost none experiment about supplementing β -glucan and vitamin E together. Three experiments were conducted to investigate the impacts of β -glucan with vitamin E supplementation in weaning pigs (Experiment 1), in growing and finishing pigs (Experiment 2), and in lactating sows (Experiment 3).

Chapter II. Review of Literature

1. Feed additives

1.1 Introduction

Antibiotics have been widely used for over 60 years in livestock breeding environments to improve growth performance and prevent numerous diseases (Anderson et al., 1999). There are growing concerns about the discovery of antibiotic-resistant bacteria in humans and livestock and the cross-infection of bacteria resistant to antibiotics between humans and livestock. Accordingly, the European Union (EU) has decided to completely ban antibiotics as growth promoters in 2006 (Casewell et al., 2003; Chen et al., 2005). In addition, Korea also has forbidden antibiotics as growth promoter from 2011. Due to the limited use of antibiotics in commercial swine farming environment, many animal scientists have tried to find natural alternatives (Park et al., 2018).

1.2 Alternative feed additives for antibiotic in swine diets

1.2.1 Acidifiers

Acidifiers are compounds with acidic properties, which can be organic or inorganic acids (Partanen and Mroz, 1999; Papatsiros and Billinis, 2012). They are often used as an alternative to antibiotic growth promoters because they can create a favorable intestinal environment for beneficial microbes, thus increasing nutrient digestibility, increasing growth performance, and reducing diarrhea. Organic acids include formic, fumaric, lactic, benzoic, propionic, and citric acids. Inorganic acids include hydrochloric, sulfuric, and phosphoric acids. Blends of acidifiers may also be used to maximize acidification effects in diets for pigs (Zentek et al., 2013; Kuang et al., 2015).

However, despite many years of research, the exact mechanism of action of

dietary acidifiers has not been fully elucidated, but the following mechanism has been proposed. Acidifiers are believed to enhance growth performance through a decreased or stabilized gastric pH of the digestive tract, which improves nutrient digestibility in the small and large intestines and promotes growth of beneficial bacteria while inhibiting pathogenic bacteria (Jacela et al., 2009a).

Acidifiers are generally targeted for weaning pigs, but have a positive effect on growing and finishing pigs as well as sows. Organic acids have been shown to improve the growth performance of weaning pigs more consistently than inorganic acids. Growth rate and feed conversion ratio were improved when formic acid, citric acid, and benzoic acid were included in diets fed weaning pigs (Diao et al., 2016; Luise et al., 2017). Acidifiers show positive effects on growing and finishing pigs, particularly under transition or stressful conditions (Tung and Pettigrew, 2006) and improve the apparent digestibility of Ca and P in growing pigs (Xu et al., 2018). In sows, use of citric acid and benzoic acid has been shown to improve nutrient digestibility (Liu et al., 2014 a, b; Kluge et al., 2010). In case of inorganic acids, they can be considered as an alternative for organic acids because of lower cost.

1.2.2 Prebiotics

Prebiotics have been described as food/feed substances that benefit the host by selectively stimulating growth or activity of favorable bacterial species in the gut (Gibson and Roberfroid, 1995). Prebiotics are primarily derived from nondigestible oligosaccharide compounds. Oligosaccharide compounds should be resistant to digestion and absorption. They also provide readily nutrient substrates for normal bacteria to grow and can be used to selectively promote colonization of acid-producing bacteria.

Oligofructose, fructooligosaccharide, and inulin are typical examples that have been used as prebiotics. They are considered carbohydrates that are easily fermented by beneficial bacteria in the hindgut. The beneficial effects of prebiotics in swine diets are associated with increased fermentability, because there is a decrease in intestinal pH due to subsequent short chain fatty acid (SCFA) synthesis. Increased concentrations

of SCFA also decrease protein fermentation in the intestinal (Lindberg, 2014). Prebiotics have been shown to be effective against pathogenic bacteria in pigs (Tran et al., 2016). Most of the prebiotic effects were consistent at the gut level (van der Aar et al., 2017). Supplementing 100 or 200 mg/kg of chitooligosaccharide in weaning pig diets improved growth performance, increased nutrient digestibility, and decreased the incidence of diarrhea (Liu et al., 2008). Isomaltooligosaccharide added to weaning pig diets at 6g/kg improved growth performance, reduced fecal scores, and increased nutrient digestibility. Also, the addition of isomaltooligosaccharides to the diet resulted in greater villi height in the ileum and increased volatile fatty acid concentrations in the cecum and colonic contents (Wang et al., 2016 a, b; Wu et al., 2017). In addition, several studies have also shown the immunity effects. Prebiotic lactulose may increase serum IgM and IgA concentrations and enhance immunity against *Salmonella typhimurium* (Naquid., 2015). Similarly, increased cell-mediated immune response, IL-1b gene expression, and serum levels of IL-1b, IL-2, and IL-6, were observed when chitosan and galacto-mannan oligosacch were supplemented for weaning pigs (Yin et al., 2008).

1.2.3 Direct-fed microbials

Direct-fed microbials, more commonly known as DFM or probiotics, are defined as, “live microorganisms that can beneficially affect the host animal by improving desirable microbiota balance within the small and large intestine (FAO/WHO. 2001). Direct-fed microbials are generally categorized into 3 main groups : *Bacillus*, lactic acid-producing bacteria, and yeast (Stein and Kil, 2006; NRC, 2012). *Bacillus*-based DFM are spore-forming bacteria. They are thermostable and can survive at low pH. *Bacillus*-based DFM can improve nutrient digestion and utilization since they have been identified as potent producers of extracellular fiber-degrading enzymes (Ferrari et al., 1993). Lactic acid-producing bacteria are not spore-forming and cannot survive during feed processing because they are not thermostable. Lactic acid-producing bacteria include *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Enterococcus faecium*. They can help lower the pH in the gut by producing lactic acid

through fermentation, suppressing enteric pathogens (Vandenbergh, 1993) and improving host immunity (de Lange et al., 2010).

For direct-fed micorbials to be effective, the microbes must survive and flourish in the gut environments. These mixtures should work by either directly excluding harmful bacteria or by indirectly helping the development of other desirable health-promoting microbes that compete with harmful bacteria with the reduction of intestinal pH. The suggested benefits of DFM are to improve weight gain and feed efficiency by improving digestion, stimulating of gastrointestinal immunity, and increasing resistance to intestinal infectious diseases (Kenney et al., 2011; Cromwell, 2013). According to Liu et al (2018) experiment, lactic acid-producing bacteria was beneficial for weaning pigs, helping to balance the gut microbiome after weaning, while *Bacillus*-based DFM was more beneficial to growing and finishing pigs to increase the digestibility of energy and nutrients in high-fiber diet.

1.2.4 Yeast

Yeast cultures are considered as direct-fed microbials and the most commonly used in swine diets include *Aspergillus oryzae*, *Candida pintolopesii*, *Saccharomyces boulardii*, and *Saccharomyces cerevisiae*. Yeasts may be supplemented in diets for pigs in many forms such as whole live yeast cells, heat-treated yeast cells, ground yeast cells, purified yeast cell cultres, and yeast extracts but they are mainly used as live yeast cultures or yeast derivatives like yeast cell walls. Yeast has many advantages when they supplemented to pig feed. They increase growth performance, and mucosal immunity, promote intestinal development, absorb mycotoxins, reduce post-weaning diarrhea, and modulate gut microbiota (Kogan and Kocher, 2007; Jiang et al., 2015).

The main point for yeast is to improve in resistance against infections and modulate immunity. Yeast cell walls seem to enhance immunity through the stimulation of immune cell function, upregulation of cytokines, and antioxidant activity (Kogan and Kocher, 2007). They also can have the toxic binding capacity, which is mainly explored as mycotoxins adsorbents. According to many previous

experiments, immunity and growth performance of nursery pigs were improved by yeasts (Shen et al., 2009; Kiarie et al., 2011; Kiros et al., 2018),

1.2.5 Plant extracts

Plant extracts are defined as compounds of plant origin that are incorporated into an animal's diet to promote growth performance and product quality. According to origins and processes, they are classified as follows: extracts, spices, essential oils, and oleoresins (Windisch et al., 2007). Modern laboratory techniques allow the isolation and characterization of bioactive substances from plant sources. The compounds range from capsaicin (cayenne pepper), cinnamaldehyde (cinnamon), eugenol (cloves), and carvacrol (oregano). In addition, the content of active substances in each compound varies greatly depending on the herbal part (seed, leaf, stem, root, or bark), geographical origin, herb maturity stage, preservation method, period, storage, and extraction method (Windisch et al., 2007).

Plant extracts show various effects such as growth performance improvement, antimicrobial activity, influence on diet palatability and gut functions, antioxidant activity and so on (Baydar et al., 2004; Dundar et al., 2008; Stein and Kil, 2006). Allan and Bilkei (2005) reported that supplementing 1,000 mg/kg oregano extract had lower annual sow mortality rate, lower sow culling rate, increased farrowing rate, and decreased stillbirth rate. They also reported that 1,000 mg/kg oregano extract increased average daily weight gain and decreased disease incidence of weaning pigs. According to Cullen et al. (2005) and Janz et al. (2007), growing and finishing pigs fed with a garlic-treated diet had higher ADG, ADFI, and feed efficiency compared to the control. In addition, plant extracts have been anti-microbial, anti-inflammatory, and to be used as antioxidant. For example, plant extracts exhibit broad antibacterial activities against gram-negative and gram-positive bacteria (Wong et al., 2008). Plant extracts inhibit the production of pro-inflammatory cytokines and chemokines from endotoxin stimulated immune cells and epithelial cells (Liu et al., 2012). Plant extracts protect animals from oxidative damage caused by free radicals (Liu et al., 2018).

1.2.6 Medium chain fatty acids

Medium-chain fatty acids (MCFA) are saturated fatty acids with 6 to 12 carbons in length, and are caproic (C6:0, hexanoic acid), caprylic (C8:0; octanoic acid), capric (C10; decanoic acid), and lauric (C12; dodecanoic acid) acids. MCFA are fully saturated and unbranched monocarboxylic acids. They are also composed of C2 units (acetyl-CoA) and they have an even number of carbon atoms. MCFA are found in various feed ingredients, especially coconut oil and palm oil. MCFA can be easily digested and rapidly absorbed, which makes MCFA a readily available source of energy for the pigs (Zentek et al., 2011). MCFA also have antibacterial effects to inhibit intestinal microbial counts which can help piglets improve growth performance (Yen et al., 2015).

There were many experiments about the inclusion of MCFA in swine diets which have shown to improve growth performance and gut health (Zentek et al., 2011; Li et al., 2015). Unique physiological and biological properties of MCFA resulted in improved growth performance of weaning pigs (Li et al., 2015). Weaning pigs could digest and absorb MCFA easily which could be effectively used as energy for growth performance.

2. β -glucan

2.1 General characteristics of β -glucan

β -glucans are a group of naturally occurring polysaccharides and are glucose polymers having a common structure comprising a main chain of β -(1,3)-glucopyranosyl units with side chains of various branches and lengths (Han et al., 2020). β -glucans are biologically active compounds which helped to improve immunity for human and animals (Wang et al., 2017). β -glucan initiated an immune response through immune cells that are activated by the binding of a polymer to a specific receptor (Han et al., 2020). However, depending on sources of β -glucan, they also have different structures, morphology, physical properties, polymer charge and

thus biological function. All these characteristics can affect their immuno-modulatory effects.

2.2 History of β -glucan

The history of β -glucan, which is typically present in the cell wall of yeast, mushrooms, and grains, goes back to the past. Some said that lentinan, isolated from shiitake mushroom, was an example of a pharmaceutically formulated polysaccharide used for medicinal purpose in China and Japan for thousands of years ago (Zhang et al., 2018). Other said that the proper history of polysaccharides as immunomodulators dated back to the 1940's, when Shear and coworkers described substances that induce tumor necrosis in *Serratia marcescens* cultures (Shear et al., 1943). This material was then identified as a mixture of three polysaccharides with a backbone composed of D-glucose and D-mannose units linked by (1 \rightarrow 3) glycosidic bonds (Srivastava and Adams, 1962).

Additional studies have been done on polysaccharide immunomodulators, including β -glucan over time. The research was started mainly in Europe and America, and then in Asia, mainly in Japan. β -glucans were discovered unintentionally through the discovery of Properdin in the complement system. The study of β -glucans in a western setting was based on knowledge of the immunomodulatory effects of zymosan, a mixture of polysaccharide isolated from the cell wall of *Saccharomyces cerevisiae*. Pillemer and Ecker (1941) firstly manufactured and investigated Zymosan in the 1940s, and used it in many physiological and immunological studies since that time. Zymosan is an activator of phagocytes cells that increases lysosomal enzyme secretion levels, upregulates leukotriene production in monocytes, and enhances the release of proinflammatory cytokines.

Although Zymosan was able to stimulate non-specific immune responses, but it was not initially clear which component of this rather crude composition was responsible for its activity. When zymosan was examined in detail by Diluzio and Riggi, β -glucan was identified as the component of primary effect (DiLuzio and Riggi, 1970; Williams et al., 1980)

The appearance of β -glucan in Japan was different. In Asia, there has been a long tradition of using various medicinal mushrooms from the past. In detailed studies of the biological effects of these mushrooms, particularly its anticancer activity, β -glucan was again identified as a major contributor to non-specific immune modulation. Goro Chihara from Teikyo University in Kawasaki, who isolated β -glucan named by him lentinan from mushroom shiitake, found this source (Chihara et al., 1969). To date, β -glucan isolated from *Lentinus edodes* called Lentinan and Polysaccharide K are the two approved drugs in Japan (Ina et al., 2013).

In 1987, Dr. William Browder, research director at Tulane University in New Orleans, said "Clinical use of immunomodulators can alter conventional use and dosage of antibiotics". He conducted the experiment and suggested that β -1,3-glucan can reduce the amount of conventional antibiotics needed for infectious conditions such as peritonitis. A combination of β -1,3-glucan and a standard antibiotic increased the long-term survival by 56% in mice which were infected with a bacteria to produce peritonitis. Bacterial counts were noticeably reduced within 8 hours of injection, and the number of key immune cells was significantly increased (Beta glucan research, 2021).

The significant heterogeneity of all natural β -glucans from multiple sources has been, and continues to be, the cause of a series of contradictory conclusions. Therefore, continuous and diverse research on β -glucan should be actively conducted.

2.3 Sources, structures, and functionalities of β -glucan

β -glucans are groups of polysaccharides or dietary fibers composed of D-glucose monomers linked by (1 \rightarrow 3), (1 \rightarrow 4) or (1 \rightarrow 6) glycosidic bonds (Van Steenwijk et al., 2021). β -glucans are polysaccharides occurring naturally in various organic sources such as bacteria, fungi, algae, and cereals such as oat and barley (Michela et al., 2014; Figure 1).

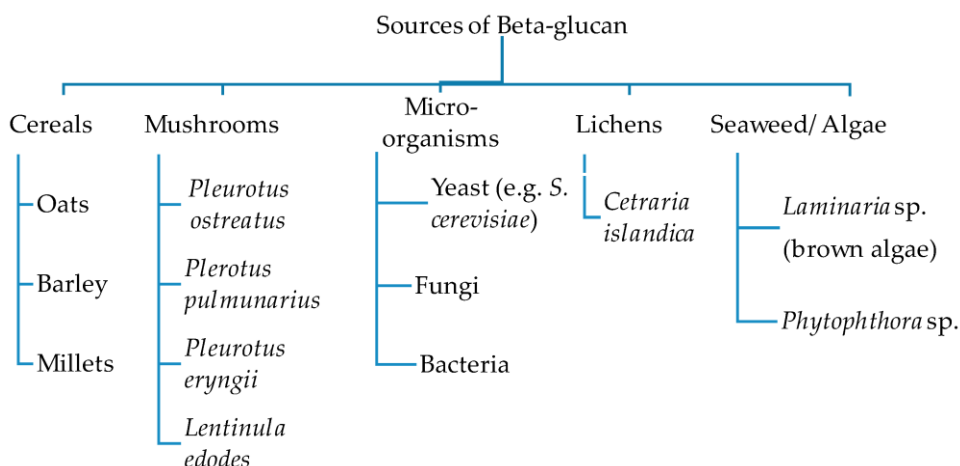


Figure 1. Sources of β -glucan

However, different sources of β -glucan also differ in the D-glucose monomers linked through β -glycosidic bonds (Figure 2). Cereal-derived β -glucans are primarily mixtures of β -(1 \rightarrow 3) and β -(1 \rightarrow 4) glycosidic linkages without β -(1 \rightarrow 6) linkages (Nakashima et al., 2018). Yeast and fungal β -glucans contain long β -1,6 and short β -1,6 branches respectively, whereas bacterial β -glucans have straight and unbranched β -(1 \rightarrow 3)-D-glucan backbones (Manners et al., 1973; Volman et al., 2008).

β -glucan type	Structure	Description
Bacterial		Linear β 1,3-glucan (i.e. <i>Euglena gracilis</i>)
Fungal		Short β 1,6 branched β 1,3-glucan (i.e. <i>Schizophyllum commune</i>)
Yeast		Long β 1,6 branched β 1,3-glucan (i.e. Black yeast)
Cereal		Linear β 1,3 / 1,4-glucan (i.e. Barley)

Figure 2. The structure and description of four kinds of β -glucans

In addition, the molecular weights, solubility, and viscosity causing various physiological functions are distinct depending on the type of β -glucans (Lee et al., 2015). β -glucan is well known for modulating immune functions such as

phagocytosis, having anticancer and antibiotic properties, and lowering blood pressure or cholesterol levels (Rahar et al., 2011).

2.4 β -glucan as an immune modulator

One important supplement that has been studied for immunological effects was β -glucans. β -glucans are well known for their ability to stimulate immune system, increase resistance to diverse viral, bacterial, protozoan, and fungal diseases as well as promote anti-tumor activity (Murphy et al., 2010). There were many research about immune modulating effects of β -glucan on pigs. Supplementation of pig diets with β -glucans has been demonstrated to boost the immune system (Mao et al., 2005; Leonard et al., 2012, Luo et al. 2019; Luo et al., 2020)

In the Leonard et al. (2012) study, researchers conducted the experiment to find out the effects of maternal dietary supplementation with β -glucan (0 vs 10 g/d) from d 107 of gestation until weaning (d 26) on immunity. Sows supplemented with β -glucan had higher colostrum IgA ($P < 0.01$) and had a trend for greater IgG ($P = 0.062$) concentrations compared with non- β -glucan supplemented sows. Piglets which suckled β -glucan supplemented sows had higher serum IgG ($P < 0.05$) concentrations on d 14 of lactation compared with those who suckled non- β -glucan supplemented sows. In Luo et al. (2019) experiment, there were five dietary treatments supplemented with 0, 25, 50, 100, and 200 mg/kg β -glucan. Increases in IL-10 (linear; $p < 0.05$) and the decline in IL-2 (linear; $p = 0.05$) and TNF- α ($p = 0.07$) were found in the jejunum of pigs supplemented with 100 mg/kg β -glucan. The other experiment was conducted to verify the immune-modulatory effects of two types of 50 mg/kg⁻¹ β -glucans (average molecular weights of HG and LG were 2000 kDa and 300 kDa, respectively) in LPS-induced weaning pigs (Luo et al. 2020). Supplementation with low and high molecular weights of β -glucans decreased the production the production of IL-1 β and TNF- α and increased IL-10 production, which is likely associated with key factors such as TLR4 and NF- κ B. This meant that supplementing β -glucan decreased concentrations of pro-inflammatory IL-1 β and TNF- α cytokines and increased the anti-inflammatory cytokine IL-10 after LPS stimulation. According to

Mao et al. (2005) experiment, they designed a 2 x 3 factorial arrangement; the level of β -glucan (0, 500, 1000 mg/g; as fed basis) and presence of immunological challenge (with or without LPS). When 500 mg/kg of β -glucan was supplemented, the release of inflammatory cytokines and corticosteroids were reduced and IL-2 bioactivity was improved to improve the lymphocyte proliferative response of weaning pigs.

As in the previous study above, β -glucan acts as an immune modulation to increase resistance against pathogens. β -glucan plays an important role in the activation of the innate immune system. They stimulate the immune system and increase resistance to various viral, bacterial, protozoan, and fungal diseases as well as promote antitumor activity (Murphy et al., 2010). The potential modes of action for the immunomodulatory effects of β -glucan were not yet fully understood, but the involvement of dectin receptors in many studies has been reported (Kim et al., 2019). Dectin which was a major β -glucan receptor for several immune cells could find out and bind β -glucan from a variety of sources (Goodridge et al., 2009). This recognition may transmit intracellular signals, activate adaptive anti-microbial and anti-fungal activities to enhance disease resistance (Brown et al., 2003; Taylor et al., 2007; Goodridge et al., 2009). In addition, β -glucan could decrease secretion of pro-inflammatory cytokines and increase secretion of anti-inflammatory cytokines to distribute the nutrients between immune system and growth effectively.

2.5 β -glucan to improve growth performance

There were many research about the effects of β -glucan on the growth performance in many phases including sows, weaning pigs, growing and finishing pigs. Supplementation of pig diets with β -glucans has been demonstrated to improve growth performance (Szuba-Trznadel et al., 2014; Lee et al., 2017; Park et al., 2018; Luo et al., 2019).

In the Szuba-trznadel et al. (2014) study, yeast derived β -glucan was added by each level (0%/0.01%/0.02%/0.03%). Supplementing 0.02% β -glucan in lactating sow diets increased average daily feed intake by sows during lactation ($p < 0.05$), body

weight of piglets on the 45th day ($p < 0.05$), daily gain of piglets during 2nd to 45th day ($p < 0.05$) and improved feed conversion ratio during 21st to 45th day significantly compared to the control ($p < 0.01$). Park et al. (2018) conducted the experiment with 150 weaning pigs to determine the effects of β -glucan on growth performance. There were five different corn-soybean meal based dietary treatments : 1) antibiotic (30 ppm, Tiamulin), 2) 0% β -glucan, 3) 0.1% β -glucan, 4) 0.2% β -glucan, 5) 0.4% β -glucan. As a result, supplementing β -glucan linearly increased ADG during phase 1 and overall period ($p < 0.01$), and improved FCR during phase 1 ($p < 0.01$), phase 2 and overall period ($p < 0.05$). According to Lee et al. (2016) experiment, 75 weaning pigs were used in the experiment which were fed (1) a corn-soybean meal-based control, (2) 0.1% β -glucan from mulberry leaves, and (3) 0.1% β -glucan from curcuma. Weaning pigs fed β -glucans from mulberry leaves and curcuma had higher average daily gain and gain/feed intake ratio than control ($p < 0.05$). Luo et al. (2019) conducted the experiments with 96 growing and finishing pigs. There were four treatments including 1) control, 2) basal+50 mg/kg β -glucan 3) basal+100 mg/kg β -glucan, 4) basal+200 mg/kg β -glucan. As a result, pigs fed with 100 mg/kg β -glucan significantly showed higher ADG and lower FCR compared to the control ($p < 0.05$).

In addition to the previous studies above, there were many results on the improvement of growth performance by the addition of β -glucan in pig feed in other studies (Dritz et al. 1995; Hahn et al. 200; Li et al. 2006). However, the exact mechanism for the improvement of growth performance by the addition of β -glucan in pig feed was precisely unknown (Vetvicka et al, 2014; Vetvicka and Oliveira 2014). However, β -glucan has been shown to improve growth performance by promoting gut health and enhancing body immunity in several other animal experiments, including rats (Belobrajdic et al., 2015), chickens (Tian et al., 2016), fish (Jiang et al., 2016), and cattle (Ma et al., 2015). In addition, β -glucan is a kind of natural immune enhancer, which strengthens the mucosal barrier function and improves the gastrointestinal environment. This helps to digest nutrients in the stomach and to improve absorption into the body through the intestinal mucosa, contributing to the growth of animals (Vetvicka et al., 2014; Luo et al., 2019).

Considering these points, the addition of β -glucan on swine diet has a positive effect on the growth performance of pigs, and further study on the detailed mechanism is needed.

3. Vitamin E

3.1 Forms and structures of vitamin E

Vitamin E is a plant-derived, lipid-soluble substance which exists in eight different forms, four tocopherols (α , β , γ , δ) and four tocotrienols (α , β , γ , δ). All feature a chromanol ring with a hydroxyl group that can donate a hydrogen atom to reduce free radicals and a hydrophobic side chain that can penetrate biological membranes. Both tocopherols and tocotrienols occur in alpha, beta, gamma, and delta forms, which are determined by the number of methyl groups in the chromanol ring (Burton and Ingold 1981). One form, α -tocopherol, is the most abundant form in nature (Sheppard et al., 1993) and is the most biologically active form (Bieri and McKenna, 1981) and its structure is in Figure 3.

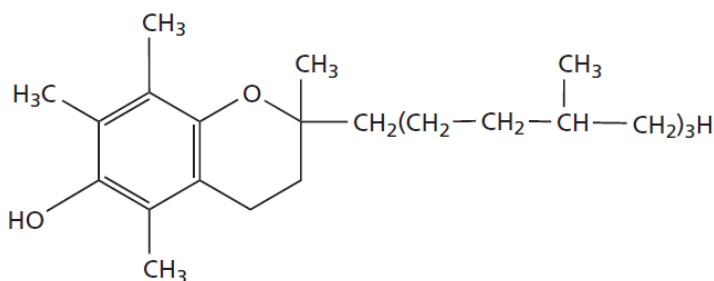


Figure 3. The structure of α -tocopherol

3.2 Vitamin E as an antioxidant

One of the main functions of vitamin E (α -tocopherol) is to prevent the propagation of free radical reactions as an antioxidant that breaks chains in biological

membranes (Combs, 1998; Surai, 2001). α -Tocopherol, the most biologically active and abundant form of vitamin E in vivo, efficiently transfers hydrogen atoms to lipid free radicals such as peroxy, alkoxy and carbon-centered radicals, providing a corresponding non-radical product of the lipid and α -tocopheroxy radicals. Once formed, the α -tocopheroxy radicals react with a second free radical or with each other to form a non-radical product. Each molecule of α -tocopherol consumes two lipid free radicals and terminates the free radical chain reaction (Yamauchi, 1997). The antioxidant activity of tocopherol is related to scavenge the free radicals of unsaturated lipids and prevent the oxidation of lipids within membranes (Burton and Ingold, 1981). Direct cellular damage can occur if the lipid hydrogen peroxide is formed in the absence of an adequate supply of tocopherols (Duthie and Arthur, 1991). Vitamin E functions as a chain-breaking antioxidant that neutralizes free radicals and prevents intra-membrane lipid oxidation (Burton et al., 1983; Ingold et al., 1987; Packer, 1994; Kamal-Eldin and Appelqvist, 1996). The consequences of lipid peroxidation include membrane damage, inhibition of enzyme activity, and accumulation of reaction products (Ullrey, 1981). There is a clear relationship between the biological and antioxidant activity of tocopherols (Combs, 1998).

The role of the antioxidants of vitamin E in pigs varied according to the growth stage of pigs. In the period of neonatal and nursing pigs, the only source of α -tocopherol for newborn pigs is the milk intake through mammary secretions. The low neonatal tissue concentrations of tocopherols place the neonatal pigs in a very poor antioxidant state against free radical damage (Mahan, 1994). After all, the best way to provide a source of antioxidant vitamins to newborns and lactating pigs is through proper nutrition of sows. This is because the amount of α -tocopherol in the colostrum and later milk consumed by newborn pigs differs depending on the amount of α -tocopherol supplied to the feed of gestating and lactating sows (Mahan, 1991). The condition of free radical damage during weaning is also important stage in a pig's life. For weaning pigs, muscle and metabolic activity are rapidly increasing. Also, a considerable amount of weaning stress is being placed such as moving to a new place, living together with other pigs, new type of feed etc). Collectively, these conditions

help increase the need for the antioxidant system to function optimally. Because of the sharp increase in muscle gain during the post-weaning period, the structural conformation of the cellular lipoprotein membrane may be compromised in relation to antioxidant protection, particularly in regards to α -tocopherol and Se. Consequently, there are many factors in weaning pigs that appear to affect α -tocopherol utilization.

The role of antioxidant vitamins in the growing and finishing phase primarily reflects the need for tissue growth and potentially higher demands during periods of stress (environment and disease). Muscle accumulation increases rapidly over a period of between 20 kg and 75 kg body weight, and the protection of cells from free radical damage caused by peroxide damage relies on antioxidant nutrients. Vitamin E-selenium deficiency occurs less frequently at this age than in pigs during the post-weaning period. When free radicals are formed of intracellular or extracellular origin, α -tocopherol acts as a mechanism to prevent peroxide damage by providing H ions, preventing the formation of free radicals. Without this antioxidant protection, free radical damage occurs and cellular contents leak into the circulatory system, resulting in elevated levels of SGPT and SGOT, two indicators used to reflect cellular damage. In reproduction phase, vitamin E-deficient diets result in fetal death and absorption, but supplementation levels of vitamin E required to prevent gastric deficiency and achieve maximum fertility have been shown to be within 10-22 IU/kg (NRC, 1988; Pharazn et al., 1990). Previous research has shown that providing higher levels of vitamin E to gestating sows on a corn-soybean meal diet in a complete breeding environment not only increased litter size but also lowered reproductive disease status (Whitehair et al., 1985; Mahan, 1991, 1994). When fed to productive sows, supplementation of 44 to 66 IU/kg of vitamin E per kg diet can increase litter size. Also, feeding 44 to 66 IU of dietary vitamin E increases α -tocopherol concentrations in colostrum and sow milk.

3.3 Vitamin E as an immune modulator

There are reports that vitamin E can affect positively on some parameters of the immune system in pigs. In vitamin E deficiency, most immune parameters tend to decrease, which is associated with an increased incidence of infectious diseases and tumors. In contrast, vitamin E supplementation has a variety of beneficial effects on the host immune system. In addition, the contents of tocopherol in immune cells is known to be higher than in other cells, because the cell membrane plays an important role in the immune response (Coquette et al., 1986). However, the optimal level of vitamin E required to improve the immune system has not been determined due to several factors such as diet composition, feed consumption, animal growth rate, and living conditions or stress.

Previous study has demonstrated that supplementing vitamin E could increase resistance in the sow and the neonatal pig to enteric diseases (Pharazyn et al., 1990). Peplowski et al. (1981) also reported that adding 220 mg/kg⁻¹ vitamin E on weaning pig diets caused a significant increase in the antibody titre to a challenge with red blood cells in weaning pigs. The data suggest an effect of vitamin E on the responsiveness of the humoral immune system.

Cell-mediated immune responses to vitamin E supplementation have also been reported in pigs. There was an increased phytohaemagglutinin (PHA)-response in lymphocytes in pigs supplemented with vitamin E at a level of 40 mg/day⁻¹ and selenium at levels of 0.5 and 0.1 ppm (Larsen and Tollersud, 1981). This result showed that the group supplemented with only vitamin E gave a significantly greater response to PHA compared with the group supplemented with only selenium. In another study, a high level of vitamin E (136 mg α -tocopherol·kg⁻¹ diet) and 0.1 mg Se·kg⁻¹ in the sow diet influenced vitamin E concentration in the serum of piglets during the first days of lactation. The results suggested that there was a carryover effect during lactation from sow diets containing a high level of vitamin E about the increase in the humoral response of pigs after weaning (Babinszky et al., 1991). However, the optimal level of vitamin E needed to improve the immune system is not yet clear, and further research is needed.

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Chapter III. Effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, fecal microbiota, fecal score, and nutrient digestibility in weaning pigs

ABSTRACT: This study was conducted to evaluate effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, fecal microbiota, fecal score, and nutrient digestibility in weaning pigs. A total of 200 weaning pigs with an average body weight (BW) of 7.64 ± 0.741 kg were allotted to five treatment groups and were divided based on sex and initial BW in four replicates with ten pigs per pen in a randomized complete block design. The experimental diets included a corn–soybean meal-based basal diet with or without 0.1% or 0.2% β -glucan and 0.02% vitamin E. The pigs were fed the diets for 6 weeks. A total of 15 barrows were used to evaluate the nutrient digestibility by the total collection method. The BW and feed intake were measured at the end of each phase. Blood samples were collected at the end of each phase, and fecal samples were collected at the end of the experiment. The addition of β -glucan with vitamin E to weaning pig feed increased BW, average daily gain, and average daily feed intake. A significant decrease in yeast & mold and Proteobacteria and a tendency for *Lactobacillus* to increase compared to the control was shown when 0.1% β -glucan and 0.02% vitamin E were added. The fecal score in weaning pigs was lower in the treatments supplemented with 0.1% or 0.2% β -glucan and 0.02% vitamin E compared to the control. In addition, vitamin E was better supplied to weaning pigs by increasing the concentration of α -tocopherol in the blood of weaning pigs when 0.02% vitamin E was supplemented. However, there was no significant difference not only in the immune response but also in the nutrient digestibility. Consequently, 0.1% β -glucan with 0.02% vitamin E in a weaning pig's diet were beneficial to the growth performance of weaning pigs by improving intestinal microbiota and reducing the incidence of diarrhea.

Keywords: β -glucan; Vitamin E; Weaning Pigs; Growth Performance; Intestinal

Microbiota; Fecal score

INTRODUCTION

Antibiotics have been widely used in feed for weaning pigs to improve feed efficiency, promote growth, and reduce diseases. However, the European Union and Korea banned the use of antibiotics as feed additives for growth promotion in 2006 and 2011, respectively (Unno et al., 2015). For this reason, research on various antibiotic substitutes, such as plant extracts, probiotics, and β -glucan, has been actively conducted.

β -glucan is a complex carbohydrate extracted from mold, grains, and the cell walls of yeast. Glucans with β -1,3 and β -1,6 glycosidic bonds are major structural components of yeast and fungal cell walls, where these bonds play a role in disease defense and growth promotion (Eicher et al., 2006). β -glucan can stimulate a series of pathways that activate the immune system and enhance both innate and adaptive immune responses (Vannucci et al., 2013). β -glucan has antitumor and antibacterial activities by enhancing host immune function (Hahn, 2006). It has a beneficial effect on the growth of weaning pigs because it induces a specific immune response and increases nonspecific immunity and tolerance to oral antigens as an immune modulator (Stokes et al., 1987).

Vitamin E, which is a very important nutrient for pigs, is known to enhance immunity through antioxidant action. In particular, vitamin E, which acts as an antioxidant at the cellular level, has a structural function and performs various functions related to reproduction (Mahan, 1991). In addition, vitamin E improves the immune response to antigens by stimulating the production of lymphocytes (Tengerdy et al., 1984). Additionally, it is an important component of all membranes found in cells, including plasma, mitochondria, and nuclear membranes (Bjørneboe et al., 1990).

There are many previous studies on the effects of β -glucan and vitamin E individually on weaning pigs, but there is insufficient evidence to verify the synergistic effect of β -glucan and vitamin E on weaning pigs. Thus, it was hypothesized that the synergistic effects of β -glucan and vitamin E could improve

immunity, leading to an increase in growth performance in weaning pigs. Therefore, this study was conducted to evaluate the effects of β -glucan with vitamin E on the growth performance, blood profiles, immune response, fecal microbiota, fecal score, and nutrient digestibility of weaning pigs.

MATERIALS AND METHODS

All experimental procedures involving animals were conducted in accordance with the Animal Experimental Guidelines provided by the Seoul National University Institutional Animal Care and Use Committee (SNU-IACUC; SNU-200209-2)

Experimental animals and housing environment

A total of 200 weaning pigs ([Yorkshire×Landrace]×Duroc) with an initial BW of 7.64 ± 0.741 kg were allotted to one of five treatments considering sex and initial BW in four replicates with ten pigs per pen in a randomized complete block design. Pigs were randomly assigned to their respective treatments by the Experimental Animal Allotment Program (EAAP) (Kim and Lindemann, 2007). Pigs were housed in an environmentally controlled facility. The pens had fully concrete floors (1.54×1.96 m). Feed and water were provided ad libitum through a feeder and a nipple during whole experimental periods. The temperature was kept at 30°C during the first 7 days and lowered 1°C every week. The experimental period was 6 weeks (phase I, 0–3 weeks; phase II, 3–6 weeks). Body weight and feed intake were measured at the end of each phase to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F ratio). In addition, feed given to all piglets was recorded each day, and feed waste in the feeder was recorded at the end of each phase.

Experimental design and diet

Dietary treatments included 1) CON (corn–soybean meal (SBM)-based diet), 2) LB (corn-SBM-based diet + β -glucan 0.1%), 3) LBE (corn-SBM-based diet + β -glucan 0.1% + vitamin E 0.02%), 4) HB (corn-SBM-based diet + β -glucan 0.2%), and 5) HBE (corn-SBM-based diet + β -glucan 0.2% + vitamin E 0.02%). A corn-SBM-based diet was used as feed in this experiment, and all nutrients in the experimental diet met or exceeded the nutrient requirements of the National Research

Council (NRC) 2012 for weaning pigs. The CP was set to 6.25 times more than the standard total nitrogen in the requirement of NRC 2012 to calculate CP requirements. In the present study, β -glucan and vitamin E products were provided by E&T Company (E&T CO., Ltd, Daejeon, South Korea). β -glucan consisted of (1,3)-(1,6)- β -D-glucan and mannan. Vitamin E was in the form of vitamin E-acetate. In the case of vitamin E, 65 IU/kg was present in the vitamin premix, and 110 IU/kg of vitamin E was additionally supplemented to the LBE and HBE treatments. All nutrient contents in the feed were formulated equally, and the formula and chemical composition of the experimental diet are presented in Table 1 and Table 2. The CP content of phase I in the weaning pig feed was 20.56%, the lysine content was 1.35%, the methionine content was 0.39%, the cysteine content was 0.35%, the threonine content was 0.79%, the tryptophan content was 0.22%, the calcium (Ca) content was 0.80%, and the total phosphorus (P) content was 0.65%. The CP content of phase II in the weaning pig feed was 18.88%, the lysine content was 1.23%, the methionine content was 0.36%, the cysteine content was 0.32%, the threonine content was 0.73%, the tryptophan content was 0.20%, the Ca content was 0.70%, and the total P content was 0.60%.

Blood profiles and immune response

Blood samples were taken from the jugular vein of three pigs near the average BW in each treatment after 3 hours of fasting on the initial day and at the end of each phase to measure vitamin E, selenium (Se), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and lymphocytes. All blood samples were collected in serum tubes (SST II Advance; BD Vacutainer, Becton Dickinson, Plymouth, UK) and centrifuged at 1,957 \times g and 4 °C for 15 min (5810R; Eppendorf, centrifuge 5810R, Hamburg, Germany). Subsequently, the supernatant was separated in a microtube (AXYGEN. INC, Union City, CA, USA) and the samples of vitamin E, IL-6 and TNF- α were stored at -20 °C, while the samples of Se and lymphocytes were stored at 4 °C for analysis. Each measurement was conducted using the following analysis

machines and techniques: Se (ICP–MS (inductively coupled plasma–mass spectrometry), ICP–MS, Perkin Elmer, Germany), vitamin E (HPLC (high-performance liquid chromatography), HPLC-UVD, PerkinElmer, USA), lymphocytes (flow cytometry, ADVIA 2120, Siemens, Germany), TNF- α (fluorescence, Luminex, Millipore, USA), and IL-6 (fluorescence, Luminex, Millipore, USA).

Fecal microbiota

Measurements of microorganisms in feces were performed at the end of the experiment. Fecal samples were collected based on the BW of the pigs in the treatments, transported to the laboratory on ice, and stored in a -80 °C freezer until further analysis. One milliliter of the pretreated sample was diluted 10-fold in steps in 9 ml of sterile 0.1% peptone water, and 1 ml of sample was taken at each dilution concentration and dispensed in 3 M dry film medium to analyze aerobic bacteria, coliform, *E. coli*, lactic acid bacteria, yeast, and mold. Subsequent triplicate spread plating was performed on Petrifilm™ aerobic plate count (APC) plates, Petrifilm™ coliform count plates, and Petrifilm™ yeast and mold count plates according to the manufacturer's instructions. APC and coliform plates were incubated aerobically at 37 °C for 24 h, and yeast and mold plates were incubated aerobically at 25 °C for 72 h in an aerobic incubation chamber. Counts were recorded as colony forming units per gram (CFU/g). In addition, fecal sample deoxyribonucleic acid (DNA) was extracted for metagenomic analysis using the DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol for comparison with the culturomic approach. All bacteria isolated through 16S rRNA sequencing were identified and classified, and the microbial composition of fecal samples was analyzed through metagenomics using next-generation sequencing (NGS) technology.

Fecal score

Observations of fecal scores were made every day at 08:00 throughout the feeding trial (35 days). Data were recorded by one trained researcher for each pen. Fecal scores were given according to the condition of feces (0 = normal feces; 1 = moist feces; 2 = mild feces; 3 = watery diarrhea) (Yen et al., 2015). Slightly wet feces on the rump area were used to designate contaminated piglets. After recording the data, we cleaned away the feces by wiping off the fecal areas or the pig's butt, preparing for a new measurement the next day.

Nutrient digestibility

A total of 15 crossbred barrows, averaging 12.48 ± 0.37 kg BW, were allotted to individual metabolic crates (40×80×90 cm) in a completely randomized design with three replicates to evaluate nutrient digestibility and nitrogen retention. The total collection method was used to determine the apparent total tract digestibility of dry matter (DM), CP, crude ash, and crude fat (McCarthy et al., 1974). After a five-day adaptation period, there was a five-day collection period. To determine the first and last day of collection, 8 g of ferric oxide and chromium oxide were added to the first and last experimental diets as selection markers. During the experimental period, all pigs were fed the phase II diets twice per day, at 07:00 and 19:00, which provided three times the maintenance energy (Kim et al., 2012), and water was provided ad libitum. Collection of feces was started when ferric oxide appeared in the feces and was maintained until the appearance of chromium oxide in the feces. Urine samples were collected during the collection period in plastic containers containing 50 mL of 4 N H₂SO₄ to prevent evaporation of nitrogen prior to nitrogen retention analysis. Fecal and urinary samples were stored at -20 °C until the end of the collection period, and the feces were dried in a drying oven at 60 °C for 72 h and then ground to 1 mm in a Wiley mill (CT 193 Cyclotec; FOSS, Höganäs, Sweden) for chemical analysis, including moisture, CP, crude fat, and crude ash contents, by the Association of Official Analytical Chemists (AOAC) methods (AOAC, 1995).

Chemical analysis

The diets and feces were ground by a Wiley mill (CT 193 Cyclotec; FOSS, Sweden) and then analyzed for DM (procedure 967.03; (AOAC, 1995)), ash (procedure 923.03; (AOAC, 1995)), and ether extract (procedure 920.39; (AOAC, 1995)). The nitrogen content was analyzed using the Kjeldahl procedure with Kjeltac (Kjeltac™ 2200; Foss Tecator, Sweden) and by calculating the CP content (nitrogen×6.25; procedure 981.10; (AOAC, 1995))

Statistical analysis

All obtained data were processed by Excel 2010 first, and then analyzed by one-way ANOVA procedure using Statistical Analysis System® 9.4 TS1M7 (SAS Inst. Inc., Cary, NC, USA). Each pen was used as the experimental unit for growth performance and fecal score, while individual pigs were used as the experimental unit for fecal microbiota, blood profile, and nutrient digestibility. The orthogonal polynomial contrasts were used to determine the effects of diet (β -glucan and vitamin E against the control), β -glucan, vitamin E, as well as the interaction between β -glucan and vitamin E. Data were presented as means and their pooled standard errors. The differences were considered as statistically significant when $p < 0.05$, while $0.05 \leq p < 0.10$ was considered to indicate a trend in the data.

RESULTS AND DISCUSSION

Growth performance

The effects of β -glucan with vitamin E supplementation in the weaning pig diet on growth performance are shown in Table 3. As a result, BW at week six, ADG for the entire period of the experiment, and ADFI for phase II (3-6 weeks) were significantly higher in all treatment groups to which β -glucan or vitamin E was added compared to the control group (Diet, $p<0.05$). In addition, the treatment groups supplemented with 0.2% β -glucan compared to those supplemented with 0.1% β -glucan showed significantly higher in BW at week six (BG, $p<0.01$), and a higher trend in ADG at phase II and the overall experimental period (BG, $p=0.09$, $p=0.08$). The addition of 0.02% vitamin E significantly increased BW at week six and ADG in phase II (VE, $p<0.01$). The HB and HBE treatments with 0.2% β -glucan had significantly higher ADFI than the treatments with 0.1% β -glucan in phase II and entire experimental period (BG, $p<0.05$). Adding 0.02% vitamin E showed a significantly higher gain:feed ratio in phase II than treatments without vitamin E (VE, $p<0.05$).

Park et al. (2018) observed that supplementation with β -glucan linearly increased ADG in phase I (0–2 weeks) and the entire period (6 weeks) and linearly decreased the feed conversion ratio (FCR) in phase I (0–2 weeks), phase II (2–6 weeks), and the entire period (6 weeks) when they compared supplementation of β -glucan by level (0%/0.1%/0.2%/0.4%) in the weaning pig diet with the treatment supplemented with 0.003% antibiotic Tiamulin. Luo et al. (2019) reported that supplementing 0.01% β -glucan in the weaning pig diet increased ADG linearly and quadratically ($p<0.05$) during the entire experimental period (28 days) when β -glucan by level (0%/0.0025%/0.005%/0.01%/0.02%) was supplemented. Pigs fed 0.005% β -glucan had significantly higher ADG ($p<0.05$) during the whole experimental period (28 days) and increased ADFI ($p<0.05$) during 0~28 days and 28~35 days when treatments with 0.005% β -glucan were compared with the control (Li et al., 2006).

Pigs supplemented with 0.1% each β -glucan from mulberry leaves and curcuma had significantly higher ADG and G:F ratio than the control ($p < 0.05$) in Lee et al.'s (2016) experiment during phase I (1~14 days). On the other hand, Zhou et al. (2013) reported that there was no significant difference in the growth performance of weaning pigs when 0.01% β -glucan was fed to weaning pigs challenged with lipopolysaccharide.

Most previous studies reported that the addition of β -glucan to weaning pig feed had a positive effect on growth performance, but the exact mechanism for improvement in growth performance was not elucidated (Vetvicka et al., 2014).

However, β -glucan, which is used as a broad-spectrum immune enhancer, has contributed to increasing the growth performance of animals such as swine by strengthening the intestinal mucosa of piglets and improving the intestinal environment when it is supplemented in the weaning pig diet (Lee et al., 2017; Rey et al., 2017). The ADFI of weaning pigs was higher than that of the control because the immune and health status of weaning pigs were improved considering the results of previous studies.

Therefore, as a result of the present experiment, the addition of β -glucan with vitamin E to weaning pig feed increased BW, ADG and ADFI. Additionally, supplementation with 0.2% β -glucan and 0.02% vitamin E had a positive effect on the growth performance of weaning pigs.

Blood profiles and immune response

The effects of β -glucan with vitamin E supplementation in the weaning pig diet on blood profiles and immune response are shown in Table 4. There was an increasing trend in vitamin E concentration in the blood profiles in groups supplemented with 0.02% vitamin E (VE, $p = 0.08$). However, there was no significant difference in the blood concentrations of selenium, tumor necrosis factor- α , interleukin-6, and lymphocytes ($p > 0.05$).

Moreira and Mahan (2002) reported that there was a significant increase in the average level of vitamin E between days 7 and 35 in treatments supplemented with

vitamin E compared to the control without vitamin E when vitamin E was added by level (0 IU/20 IU/40 IU/60 IU) in a weaning pig diet ($p < 0.05$). In addition, supplementation with 250 IU vitamin E increased the concentration of vitamin E significantly on days 42 and 68 compared to the treatment supplemented with 40 IU in the study of Rey et al. (2017) ($p < 0.01$).

Various factors influence the vitamin E status of pigs before and after weaning. Neonatal piglets are born with a low α -tocopherol concentration in their tissues (Mahan, 1991). Diarrhea after weaning lowers serum α -tocopherol concentration and worsens vitamin E absorption (Hoppe et al., 1991). In addition, vitamin E deficiency occurs most frequently in weaning pigs during the first few weeks after weaning, as postweaning serum vitamin E concentrations decrease due to low feed intake and increased stress.

In the current experiment, the treatments with 0.02% vitamin E added to the weaning pig feed showed an increasing trend in vitamin E concentration at week three compared to the treatments without vitamin E. As in the previous studies of Moreira and Mahan (2002) and Rey et al. (2017), the concentration of vitamin E in the blood tended to increase with additional vitamin E supply. This means that vitamin E is being easily delivered into the piglets according to the additional vitamin E supply in the weaning pig feed. It can also be expected that vitamin E will have a positive effect on improving the antioxidant status of weaning pigs and increasing the immune response through enhanced cell protection.

In the current experiment, the addition of 0.02% vitamin E in the diet of weaning pigs had a positive effect on the vitamin E concentration in weaning pigs.

Fecal microbiota

The effects of β -glucan with vitamin E supplementation in the diet of weaning pigs on fecal microbiota, including aerobic count (AC), coliform count (CC), *E. coli*/coliform count (EC), lactic acid bacteria count (LAB), and yeast and mold count (Y&M), are shown in Figures 1, 2, 3, 4, and 5. As a result, *Lactobacillus* was

decreased in the HBE and Y&M treatments and was decreased in the LB and LBE treatments compared to the control, as shown in Figure 1 (diet, $p < 0.05$). Treatments with 0.1% β -glucan showed significantly lower Y&M compared to that of the control, as shown in Figure 2 (Diet, $p < 0.01$). Treatments with 0.02% vitamin E also showed significantly lower Y&M compared to that of the control, as shown in Figure 3 (Diet, $p < 0.05$). In addition, the number of Proteobacteria (phylum containing pathogenic microorganisms such as *E. coli*, *Salmonella*, *Shigella*, etc.) was significantly lower in the treatments with β -glucan and vitamin E than in the control, as shown in Figure 4 (diet, $p < 0.05$). According to Figure 5, pigs fed 0.1% β -glucan showed an increasing trend of *Lactobacillus* compared to that of the control.

According to a previous study by Park et al. (2018), no significant difference was found among treatments in *Lactobacillus* and *Salmonella*, but coliform bacteria decreased linearly in feces as the amount of β -glucan increased in week six when dietary supplementation of β -glucan by level (0%/0.1%/0.2%/0.4%) in the weaning pig diet was compared with the treatment with 0.003% antibiotic Tiamulin ($p < 0.05$). Additionally, *Lactobacillus* and *E. coli* in feces in week two and five were not affected by supplementation with 0.1% each of β -glucan from mulberry leaves and curcuma in the weaning pig feed (Lee et al., 2017).

In the present experiment, YM decreased when 0.1% β -glucan or 0.02% vitamin E was added to the weaning pig feed. In addition, Proteobacteria decreased compared to the control when 0.1% and 0.2% β -glucan and 0.02% vitamin E were added. Metzler-Zebeli et al. (2011) reported that supplementing β -glucan could help the composition and metabolic activity of the microbiome in the gastric cavity, cecum, and colon. In another previous study, it was reported that β -glucan, in a mixed form as a grain or concentrate, was easily fermented, decreased the number of intestinal bacteria, and increased the intestinal butyrate concentration of growing pigs (Metzler-Zebeli et al., 2010). In addition, weaning pigs fed a diet supplemented with β -glucan for two weeks after weaning had reduced susceptibility to enterotoxigenic *E. coli*, a major cause of diarrhea (Stuyven et al., 2008). No significant difference was found in the aerobic bacteria/*E. coli*/coliform bacteria. However, in the current experiment,

the decrease in YM and Proteobacteria and the tendency for *Lactobacillus* to increase when supplementing 0.1% β -glucan and 0.02% vitamin E would have benefitted pig health through improvement of the intestinal environment.

Fecal score

The effects of β -glucan with vitamin E supplementation in the weaning pig diet on fecal score are shown in Table 5. As a result of the experiment, the treatment groups to which β -glucan or vitamin E was added had significantly lower fecal scores than those of the control at week three and six (Diet, $p < 0.05$). Furthermore, treatments with 0.2% β -glucan had significantly lower fecal scores than treatments with 0.1% β -glucan at week three (BG, $p < 0.05$). Treatments with 0.02% vitamin E also significantly showed lower fecal scores than treatments without vitamin E at week three (VE, $p < 0.05$).

In general, diarrhea in weaning pigs occurs over a period of 1–2 weeks after weaning due to a change in feed and causes damage to the digestive system. In addition, diarrhea occurs due to a decrease in absorption capacity, which is affected by shortening the length of villi, increasing the depth of crypts, and decreasing the action of digestive enzymes. A decrease in the absorption capacity of the small intestine is associated with the growth of enterotoxigenic bacteria or a decrease in the fermentation of digestible nutrients in the large intestine, which causes diarrhea in weaning pigs.

According to a previous study by Park et al. (2018), no significant difference was found among treatments when dietary supplementation of β -glucan by level (0%/0.1%/0.2%/0.4%) in the weaning pig diet was compared with the treatment supplemented with 0.003% of the antibiotic Tiamulin. Lee et al. (2017) also reported that the fecal score was not affected by supplementation with 0.1% each of β -glucan from mulberry leaves and curcuma in weaning pig feed. On the other hand, the treatment supplemented with 0.0108% β -glucan showed a significantly lower fecal score compared to that of the control and the treatment supplemented with 0.0054%

β -glucan when β -glucan by level (0%/0.0054%/0.0108%) was supplemented to weaning pigs experimentally infected with a pathogenic *E. coli* ($p < 0.05$) (Kim et al., 2019).

In the current experiment, the fecal score was significantly lower when 0.1% or 0.2% β -glucan and 0.02% vitamin E were added to weaning pig feed compared to the control. Kim et al. (2018) reported that the fecal score after weaning was low due to the enhancement of the barrier function and immunity of weaning pigs by adding β -glucan. In addition, supplying additional vitamin E is important because vitamin E absorption can be greatly reduced before and after weaning for pigs with diarrhea (Hoppe, 1991). Therefore, it was assumed that the fecal score was low because of strengthening the gut integrity of weaning pigs, improving immunity, and reducing oxidative stress through antioxidant effects with supplementation of vitamin E.

Therefore, the results of the present experiment showed that the addition of 0.1% or 0.2% β -glucan and 0.02% vitamin E to weaning pig feed lowered the fecal score.

Nutrient digestibility

The effects of β -glucan with vitamin E supplementation in the weaning pig diet on nutrient digestibility are shown in Table 6. Supplementing β -glucan and vitamin E did not affect nutrient digestibility and nitrogen retention.

According to the previous study of Lee et al. (2017), treatments supplemented with 0.1% each of β -glucan from mulberry leaves and curcuma showed higher digestibility of DM and energy than those of the control over two weeks. Hahn et al. (2006) conducted an experiment with the addition of β -glucan by level (0%/0.01%/0.02%/0.03%/0.04%) in weaning pig feed. The digestibilities of DM, gross energy, CP, ether extract, Ca, and P increased linearly ($p < 0.05$) as the addition level of β -glucan increased. When dietary supplementation of β -glucan by level (0%/0.1%/0.2%/0.4%) in the weaning pig diet was compared with the treatment with 0.003% of the antibiotic Tiamulin, supplementation of β -glucan linearly increased apparent total tract digestibility of DM and energy during 1–14 and 1–42 days as the

amount of β -glucan increased from 0.1% to 0.4% (Park et al., 2018). On the other hand, there was also a study in which the addition of β -glucan in pig feed showed different results from previous studies in terms of nutrient digestibility. The addition of 0.1% β -glucan to growing pig feed had no effect on the digestibility of DM, gross energy, CP, crude ash, or P (Ko et al., 2000).

In the present study, the addition of β -glucan with vitamin E to weaning pig feed did not affect nutrient digestibility. This current study did not show an increase in nutrient digestibility, as in previous studies by Hahn et al. (2006), Lee et al. (2017), and Park et al. (2018). The results also differed from those of a previous study by Brennan and Cleary (2005), who reported that the addition of cereal mixed-linked β -(1,3)-(1,4)-d-glucan had a negative effect on the nutrient digestibility and growth performance of pigs.

Further research is needed on the effect of β -glucan obtained from brewer's yeast used in the current experiment on nutrient digestibility in weaning pigs because the structure, chemical composition, and the effect of β -glucan were different depending on the source type.

In the current experiment, the addition of β -glucan with vitamin E to weaning pig feed had no effect on the nutrient digestibility of weaning pigs.

CONCLUSION

A significant decrease in YM and Proteobacteria and a tendency for *Lactobacillus* to increase compared to the control was shown when 0.1% β -glucan and 0.02% vitamin E were added. The fecal score in weaning pigs was lower in the treatments supplemented with 0.1% or 0.2% β -glucan and 0.02% vitamin E compared to the control. In addition, vitamin E was better supplied to weaning pigs by increasing the concentration of α -tocopherol in the blood of weaning pigs when 0.02% vitamin E was supplemented. Therefore, the addition of 0.1% β -glucan and 0.02% vitamin E to weaning pig feed is thought to have a positive effect on the growth performance of weaning pigs by improving the intestinal microbial composition and reducing the occurrence of diarrhea while efficiently supplying vitamin E.

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Table 1. Formula and chemical compositions of the experimental diets during weaning phase 1 (0-3 weeks)

Items	Treatment ¹⁾				
	CON	LB	LBE	HB	HBE
Ingredient (%)					
Expanding corn	39.84	39.65	39.60	39.46	39.42
Corn	10.00	10.00	10.00	10.00	10.00
Soybean meal	34.03	34.06	34.08	34.09	34.10
Soy oil	0.34	0.40	0.41	0.46	0.47
Sweet whey powder	4.00	4.00	4.00	4.00	4.00
Lactose	8.00	8.00	8.00	8.00	8.00
L-Lysine-HCl (78%)	0.26	0.26	0.26	0.26	0.26
DL-methionine (80%)	0.10	0.10	0.10	0.10	0.10
L-threonine (99%)	0.11	0.11	0.11	0.11	0.11
MDCP	1.51	1.51	1.51	1.51	1.51
Limestone	1.26	1.26	1.26	1.26	1.26
Vitamin. Mix ²⁾	0.10	0.10	0.10	0.10	0.10
Mineral. Mix ³⁾	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Zinc oxide	0.05	0.05	0.05	0.05	0.05
β -glucan ⁴⁾	0.00	0.10	0.10	0.20	0.20
Vitamin E ⁴⁾	0.00	0.00	0.02	0.00	0.02
Sum	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)⁵⁾					
ME (kcal/kg)	3400.00	3400.00	3400.00	3400.00	3400.00
Crude protein (%)	20.56	20.56	20.56	20.56	20.56
Lysine (%)	1.35	1.35	1.35	1.35	1.35
Methionine (%)	0.39	0.39	0.39	0.39	0.39
Cysteine (%)	0.35	0.35	0.35	0.35	0.35
Threonine (%)	0.79	0.79	0.79	0.79	0.79
Tryptophan (%)	0.22	0.22	0.22	0.22	0.22
Calcium (%)	0.80	0.80	0.80	0.80	0.80
Phosphorus (%)	0.65	0.65	0.65	0.65	0.65

MDCP, mono-dicalcium phosphate; ME, metabolizable energy; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E.

²⁾ Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 11,000 IU; Vitamin D₃, 920 IU; Vitamin E, 65 IU; Vitamin K₃, 7.5 mg; Riboflavin, 8.5 mg; Calcium pantothenic acid, 37 mg; Niacin, 55 mg; D-Biotin, 0.19 mg; Vitamin B₁₂, 0.045 mg.

³⁾ Provided the following quantities of minerals per kg of complete diet: Fe, 75 mg; Cu, 32 mg; Mn, 30 mg; I, 0.25 mg; Se, 0.1 mg; Zn, 23 mg.

⁴⁾ β -glucan and vitamin E products were provided by E&T company (E&T Co, Ltd. Daejeon, South Korea).

⁵⁾ Calculated value.

Table 2. Formula and chemical compositions of the experimental diets during weaning phase 2 (3-6 weeks)

Items	Treatment ¹⁾				
	CON	LB	LBE	HB	HBE
Ingredient (%)					
Expanding corn	30.77	30.59	30.56	30.39	30.36
Corn	30.00	30.00	30.00	30.00	30.00
Soybean meal	29.68	29.70	29.70	29.74	29.74
Soy oil	0.22	0.28	0.29	0.34	0.35
Sweet whey powder	2.00	2.00	2.00	2.00	2.00
Lactose	4.00	4.00	4.00	4.00	4.00
L-Lysine-HCl (78%)	0.23	0.23	0.23	0.23	0.23
DL-methionine (80%)	0.04	0.04	0.04	0.04	0.04
L-threonine (99%)	0.04	0.04	0.04	0.04	0.04
MDCP	1.37	1.37	1.37	1.37	1.37
Limestone	1.12	1.12	1.12	1.12	1.12
Vitamin. Mix ²⁾	0.10	0.10	0.10	0.10	0.10
Mineral. Mix ³⁾	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Zinc oxide	0.03	0.03	0.03	0.03	0.03
β -glucan ⁴⁾	0.00	0.10	0.10	0.20	0.20
Vitamin E ⁴⁾	0.00	0.00	0.02	0.00	0.02
Sum	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)⁵⁾					
ME (kcal/kg)	3350.00	3350.00	3350.00	3350.00	3350.00
Crude protein (%)	18.88	18.88	18.88	18.88	18.88
Lysine (%)	1.23	1.23	1.23	1.23	1.23
Methionine (%)	0.36	0.36	0.36	0.36	0.36
Cysteine (%)	0.32	0.32	0.32	0.32	0.32
Threonine (%)	0.73	0.73	0.73	0.73	0.73
Tryptophan (%)	0.20	0.20	0.20	0.20	0.20
Calcium (%)	0.70	0.70	0.70	0.70	0.70
Phosphorus (%)	0.60	0.60	0.60	0.60	0.60

MDCP, mono-dicalcium phosphate; ME, metabolizable energy; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E.

²⁾ Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 11,000 IU; Vitamin D₃, 920 IU; Vitamin E, 65 IU; Vitamin K₃, 7.5 mg; Riboflavin, 8.5 mg; Calcium pantothenic acid, 37 mg; Niacin, 55 mg; D-Biotin, 0.19 mg; Vitamin B₁₂, 0.045 mg.

³⁾ Provided the following quantities of minerals per kg of complete diet: Fe, 75 mg; Cu, 32 mg; Mn, 30 mg; I, 0.25 mg; Se, 0.1 mg; Zn, 23 mg.

⁴⁾ β -glucan and vitamin E products were provided by E&T company (E&T Co, Ltd. Daejeon, South Korea).

⁵⁾ Calculated value.

Table 3. Effects of β -glucan with vitamin E supplementation on growth performance in weaning pigs

Items	Treatment ¹⁾					SEM	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Body weight, kg										
Initial	----- 7.64-----					0.148	-	-	-	-
Week 3	10.44	10.72	10.29	10.74	10.75	0.121	0.57	0.42	0.47	0.46
Week 6	18.76	18.90	20.38	20.32	20.93	0.231	<0.01	<0.01	<0.01	0.15
Average daily gain, g										
0-3 weeks	133.59	146.78	126.35	147.62	148.04	5.347	0.54	0.37	0.43	0.41
3-6 weeks	395.98	389.11	480.07	456.62	484.58	12.259	0.02	0.09	<0.01	0.13
0-6 weeks	264.79	268.76	303.21	301.83	316.32	6.291	0.03	0.08	0.06	0.42
Average daily feed intake, g										
0-3 weeks	254.04	268.78	239.40	267.81	257.45	7.930	0.84	0.65	0.31	0.62
3-6 weeks	624.98	636.67	652.55	703.58	705.20	10.559	0.02	<0.01	0.63	0.69
0-6 weeks	439.51	452.73	445.96	485.69	481.33	7.588	0.14	0.04	0.72	0.94
Gain : Feed ratio (G:F ratio)										
0-3 weeks	0.531	0.554	0.532	0.553	0.593	0.025	0.69	0.62	0.89	0.65
3-6 weeks	0.635	0.611	0.736	0.650	0.690	0.016	0.32	0.90	0.02	0.20
0-6 weeks	0.606	0.594	0.679	0.622	0.663	0.015	0.38	0.86	0.08	0.52

SEM, standard error of the mean; BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E.

Table 4. Effects of β -glucan with vitamin E supplementation on blood profiles and immune responses in weaning pigs

Items	Treatment ¹⁾					SEM	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Vitamin E ($\mu\text{mol/L}$)										
Initial	-----8.90-----					-	-	-	-	-
Week 3	2.00	2.35	2.95	2.13	2.73	0.271	0.51	0.65	0.08	1.00
Week 6	2.10	3.67	3.27	3.60	3.93	0.400	0.74	0.49	0.91	0.83
Selenium (μL)										
Initial	-----99.00-----					-	-	-	-	-
Week 3	125.00	105.67	127.67	130.67	120.67	4.064	0.35	0.51	0.51	0.10
Week 6	158.00	167.34	175.33	125.00	148.67	7.002	0.17	0.08	0.28	0.58
TNF-α ($\mu\text{mol/L}$)										
Initial	-----0.47-----					-	-	-	-	-
Week 3	0.18	0.20	0.21	0.20	0.23	0.201	0.99	0.99	0.90	0.95
Week 6	0.04	0.16	0.07	0.02	1.92	0.350	0.38	0.56	0.26	0.22
IL-6 ($\mu\text{mol/L}$)										
Initial	-----0.91-----					-	-	-	-	-
Week 3	1.01	1.72	1.26	0.15	1.55	0.307	0.58	0.68	0.52	0.22
Week 6	0.18	0.50	0.13	0.17	0.29	0.078	0.62	0.71	0.49	0.21
Lymphocyte (%)										
Initial	----- 54.30-----					-	-	-	-	-
Week 3	51.13	43.97	44.70	44.47	45.13	1.512	0.61	0.32	0.85	0.99
Week 6	54.80	50.07	52.53	48.65	53.80	1.769	0.87	0.52	0.34	0.73

SEM, standard error of the mean; BG, β -glucan; VE, vitamin E; TNF- α , tumor necrosis factor- α ; IL, interleukin; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E.

Table 5. Effects of β -glucan with vitamin E supplementation on the fecal scores in weaning pigs

Items	Treatment ¹⁾					SEM	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Fecal score²⁾										
Week 3	1.09	0.89	0.69	0.79	0.65	0.048	<0.01	0.04	0.04	0.69
Week 6	0.80	0.58	0.49	0.66	0.65	0.042	0.04	0.13	0.55	0.65

SEM, standard error of the mean; BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E.

²⁾ Fecal score: 0, normal feces; 1, moist feces; 2, mild diarrhea; 3, watery diarrhea.

Table 6. Effects of β -glucan with vitamin E supplementation on nutrient digestibility in weaning pigs¹⁾

Items	Treatment ²⁾					SEM	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Nutrient digestibility, %										
Dry matter	91.41	91.53	91.83	91.55	92.25	0.74	0.85	0.90	0.79	0.91
Crude protein	90.10	88.62	89.51	90.62	90.58	0.99	0.92	0.53	0.86	0.85
Crude ash	72.52	71.35	73.69	72.39	73.78	2.29	0.96	0.92	0.75	0.93
Crude fat	80.99	82.33	81.17	80.01	81.45	1.97	0.96	0.84	0.97	0.79
N-retention, g/d										
N-intake	5.13	5.13	5.14	5.13	5.16	-	-	-	-	-
N-feces	0.51	0.58	0.54	0.48	0.49	0.25	0.92	0.54	0.87	0.84
N-urine	2.35	2.26	2.12	2.24	2.10	0.19	0.18	0.83	0.10	0.97
N-retention ³⁾	2.27	2.28	2.48	2.40	2.57	0.35	0.39	0.52	0.28	0.93

SEM, standard error of the mean; BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ A total of 15 barrows (initial body weight, 12.48 ± 0.37 kg) were used.

²⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E.

³⁾ N-retention = N intake (g) - fecal N (g) - urinary N (g)

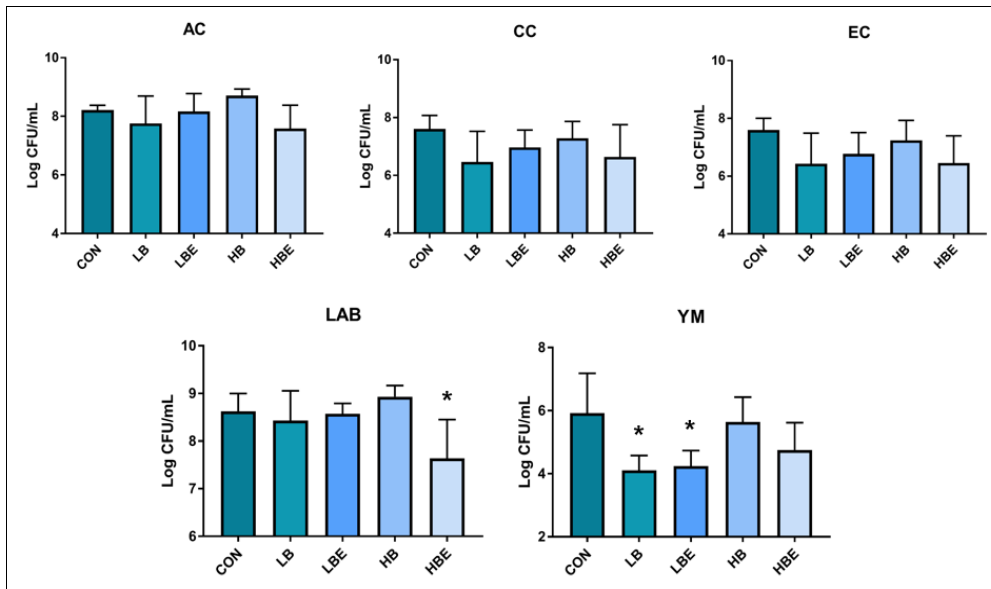


Figure 1. Fecal microbiota according to the addition of β -glucan with vitamin E in weaning pig feed (comparison of all treatments; quantitative analysis). Counts were recorded as colony forming units per gram (CFU/g). Significant differences in the figure are expressed as *, and differences were considered significant at $p < 0.05$. CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E. AC, aerobic count; CC, coliform count; EC, *E.coli*/coliform count; LAB, lactic acid bacteria count; YM, yeast and mold count.

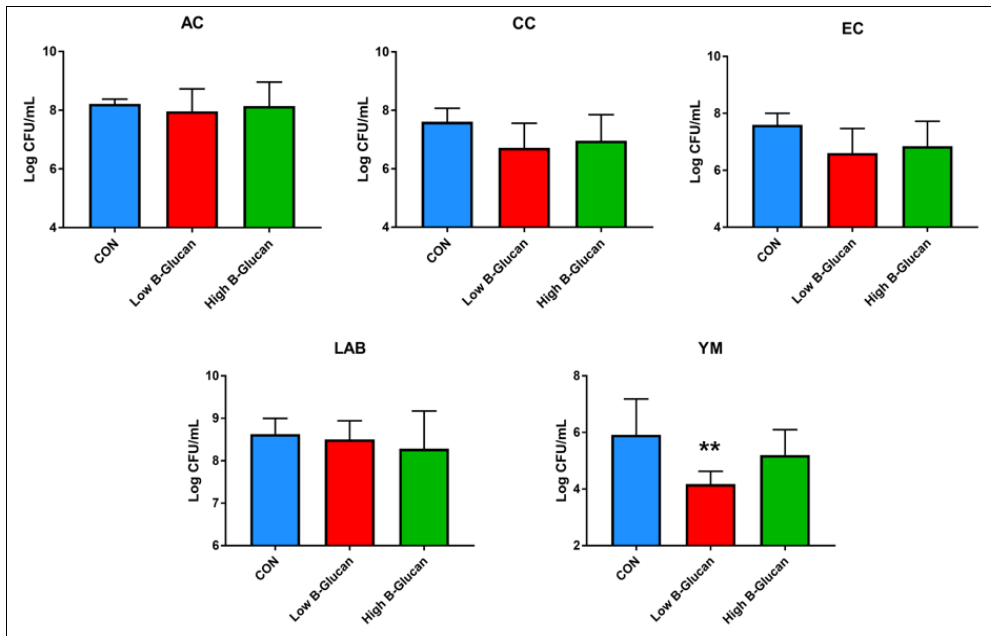


Figure 2. Fecal microbiota according to the addition of β -glucan with vitamin E in weaning pig feed (comparison according to β -glucan; quantitative analysis). Counts were recorded as colony forming units per gram (CFU/g). Highly significant differences in the figure are expressed as **, and differences were considered highly significant at $p < 0.01$. CON, corn-SBM based diet. Low β -glucan meant 0.1% β -glucan and high β -glucan meant 0.2% β -glucan. AC, aerobic count; CC, coliform count; EC, *E.coli*/coliform count; LAB, lactic acid bacteria count; YM, yeast and mold count.

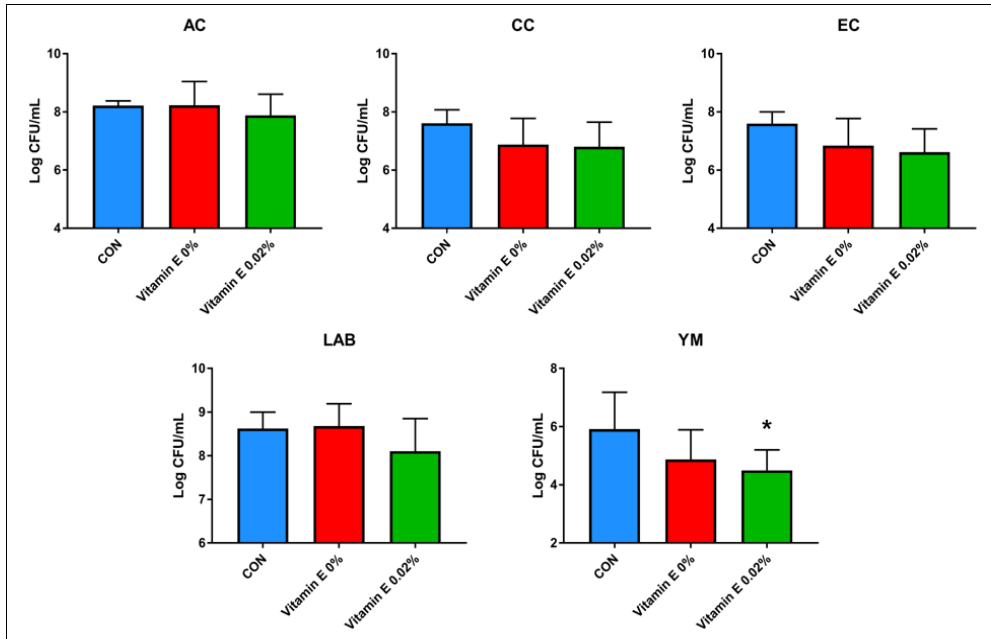


Figure 3. Fecal microbiota according to the addition of β -glucan with vitamin E in weaning pig feed (comparison according to vitamin E; quantitative analysis). Counts were recorded as colony forming units per gram (CFU/g). Significant differences in the figure are expressed as *, and differences were considered significant at $p < 0.05$. CON, corn-SBM based diet. AC, aerobic count; CC, coliform count; EC, *E.coli*/coliform count; LAB, lactic acid bacteria count; YM, yeast and mold count.

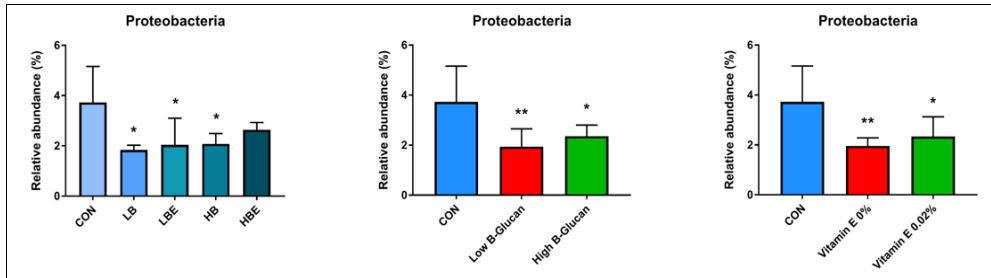


Figure 4. Fecal microbiota according to the addition of β -glucan with vitamin E in weaning pig feed (relative abundance phylum; next generation sequencing analysis). Differences were declared significant at $p < 0.05$ with * marks and highly significant differences were expressed at $p < 0.01$ with ** marks. CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E. Low β -glucan meant 0.1% β -glucan and high β -glucan meant 0.2% β -glucan.

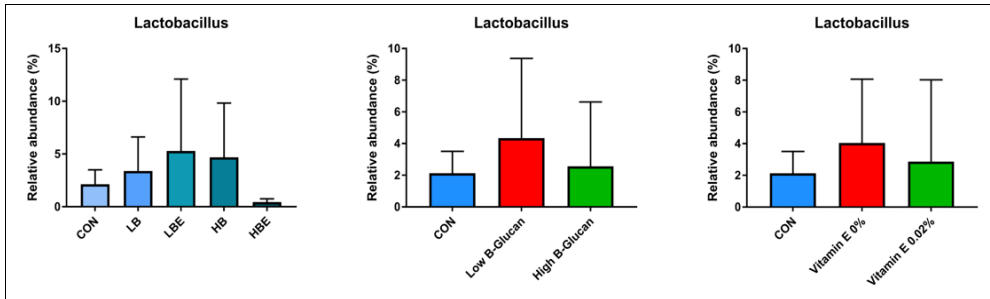


Figure 5. Fecal microbiota according to the addition of β -glucan with vitamin E in weaning pig feed (relative abundance genus; next generation sequencing analysis). CON, corn-SBM based diet; LB, corn-SBM based diet+0.1% β -glucan; LBE, corn-SBM based diet+0.1% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.2% β -glucan; HBE, corn-SBM based diet+0.2% β -glucan+0.02% vitamin E. Low β -glucan meant 0.1% β -glucan and high β -glucan meant 0.2% β -glucan.

IV. Effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, pork quality, pork flavor, and economic benefit in growing and finishing pigs

ABSTRACT: This study was conducted to evaluate the effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, pork quality, pork flavor, and economic benefit in growing and finishing pigs. A total of 140 growing pigs ([Yorkshire x Landrace]) x Duroc) were assigned to five treatments considering sex and initial BW in 4 replications with 7 pigs per pen in a randomized complete block design. The experimental diets included a corn-soybean meal based basal diet with or without 0.05% or 0.1% β -glucan and 0.02% vitamin E. The pigs were fed the diets for 12 weeks (phase I : 0-3, phase II : 3-6, phase III : 6-9, phase IV : 9-12). The BW and feed intake were measured at the end of each phase. Blood samples were collected at the end of each phase. Four pigs from each treatment were selected and slaughtered for meat quality. Economic benefit was calculated considering the total feed intake and feed price. Pork flavor was analyzed through inosine monophosphate analysis. The ADG and feed efficiency were improved compared to the control when β -glucan or vitamin E was added. Supplementing 0.05% β -glucan significantly increased lymphocyte concentration compared to the addition of 0.1% β -glucan and the content of vitamin E in the blood increased when 0.02% vitamin E was added. The HBE treatment added with 0.1% β -glucan and 0.02% vitamin E showed the most economic effect because it had the shortest days to market weight and the lowest total feed cost. The addition of β -glucan or vitamin E had a positive role in improving the flavor of pork when considering that the content of inosine monophosphate was increased. However, carcass traits and meat quality were not affected by β -glucan or vitamin E. The addition of 0.1% β -glucan and 0.02% vitamin E in growing and finishing pig's diet showed great growth performance and economic effects by supplying vitamin E efficiently and by improving the health condition of pigs due to β -glucan.

Keywords: β -glucan; Vitamin E; Growing and Finishing Pig; Growth Performance; Economic Benefit

INTRODUCTION

Antibiotics have been widely used to improve growth performance and prevent disease in livestock environments for a long time. However, the use of antibiotics is harmful to animal and human and causes serious problems such as bacterial resistance to antibiotics, presence of drug residues in pork products, and environmental pollution, etc (Du et al., 2015). Therefore, the European Union and Korea banned the use of antibiotics as feed additives for growth promotion in 2006 and 2011, respectively. For this reason, research on various antibiotic substitutes, such as plant extracts, probiotics, and β -glucan, has been actively conducted (Park et al., 2018).

β -glucan is a type of functional polysaccharide and is a component contained in the mushroom, grain seeds (oat, rye, barley, etc), and the cell wall of yeast. It has various biological functions, such as improving immune function, preventing disease, and controlling blood glucose (Xiong et al., 2015). β -glucan can stimulate a series of pathways that strengthen the immune system and enhance both innate and acquired immunity (Vannucci et al., 2013). An active immune system can help animals fight disease-causing organisms and maintain clinical infection control and growth processes. In addition, β -glucan has beneficial effects on improving growth performance, nutrient digestibility and carcass traits when it is added to growing and finishing pig feed (Luo et al., 2019).

Vitamin E, which acts as an antioxidant at the cell membrane level, has a structural function and performs several functions related to reproduction (Mahan, 1991). Adding a high vitamin E content in the growing and finishing pig feed increases the immune response (Wuryastuti et al., 1993). Vitamin E can prevent oxidized phospholipids in cell membranes and fats in feed including unsaturated fatty acids (Drochner, 1976). In addition, vitamin E mainly maintains cells and helps to minimize the damage caused by oxidative reactions in cells (Anderson et al., 1995).

There have been many previous studies evaluating the effects of β -glucan and vitamin E on growing and finishing pigs, but there is insufficient evidence to verify the synergistic effect of β -glucan and vitamin E on growing and finishing pigs.

Therefore, this study was conducted to evaluate the effects of β -glucan and vitamin E on the growth performance, blood profiles, immune response, pork quality, pork flavor, and economic benefit in growing and finishing pigs.

MATERIALS AND METHODS

All experimental procedures involving animals were conducted in accordance with the Animal Experimental Guidelines provided by the Seoul National University Institutional Animal Care and Use Committee (SNUIACUC; SNU-200209-2)

Experimental animals and management

A total of 140 growing pigs ([Yorkshire × Landrace] × Duroc) with initial body weight (BW) of 34.43 ± 2.362 kg were assigned to one of five treatments considering sex and initial BW in four replicates with seven pigs per pen in a randomized complete block design. Pigs were randomly allotted to their respective treatments by the experimental animal allotment program (EAAP) (Kim, 2007). Pigs were housed in an environmentally controlled facility. The pens were fully concrete floored (2.60×2.84 m²) and equipped with a feeder and, water nipple. The experimental period was 12 weeks (phase I : 0-3, phase II : 3-6, phase III : 6-9, phase IV : 9-12).

Experimental design and diet

Dietary treatments included 1) CON (corn–soybean meal (SBM)-based diet), 2) LB (corn-SBM-based diet + 0.05% β-glucan), 3) LBE (corn-SBM-based diet + 0.05% β-glucan + 0.02% vitamin E), 4) HB (corn-SBM-based diet + 0.1% β-glucan), and 5) HBE (corn-SBM-based diet + 0.1% β-glucan + 0.02% vitamin E). A corn-SBM-based diet was used as feed in this experiment, and all nutrients in the experimental diet except crude protein (CP) met or exceeded the nutrient requirements of the National Research Council (NRC) 1998 for growing and finishing pigs. The CP was set to 6.25 times more than the standard total nitrogen in the requirement of NRC (2012) to calculate CP requirements. In the present study, β-glucan and vitamin E products were provided by E&T Company (E&T CO., Ltd, Daejeon, South Korea). β-glucan consisted of (1,3)-(1,6)-β-D-glucan and mannan, and vitamin E was in the

form of vitamin E-acetate. In the case of vitamin E, 35 IU/kg was present in the vitamin premix, and 110 IU/kg of vitamin E was additionally supplemented to the LBE and HBE treatments. All nutrient contents in the feed were formulated equally, and the formula and chemical composition of the experimental diet are presented in Table 1, Table 2, Table 3, and Table 4.

The CP content of phase I (0-3 weeks) in the growing pig feed was 15.69%, the lysine content was 0.83%, the methionine content was 0.25%, the cysteine content was 0.27%, the threonine content was 0.60%, the tryptophan content was 0.15%, the calcium (Ca) content was 0.66%, and the total phosphorus (P) content was 0.56%. The CP content of phase II (3-6 weeks) in the growing pig feed was 13.75%, the lysine content was 0.67%, the methionine content was 0.23%, the cysteine content was 0.24%, the threonine content was 0.52%, the tryptophan content was 0.15%, the Ca content was 0.59%, and the total P content was 0.52%. The CP content of phase III (6-9 weeks) in the finishing pig feed was 12.13%, the lysine content was 0.66%, the methionine content was 0.21%, the cysteine content was 0.21%, the threonine content was 0.46%, the tryptophan content was 0.12%, the calcium (Ca) content was 0.52%, and the total phosphorus (P) content was 0.47%. The CP content of phase IV (9-12 weeks) in the finishing pig feed was 10.43%, the lysine content was 0.52%, the methionine content was 0.19%, the cysteine content was 0.20%, the threonine content was 0.38%, the tryptophan content was 0.10%, the Ca content was 0.46%, and the total P content was 0.43%.

Growth performance

Body weight (BW) and feed intake were measured at the end of each phase to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F ratio). In addition, feed given to all growing and finishing pigs was recorded each day, and feed waste in the feeder was recorded at the end of each phase.

Blood profiles and immune response

Blood samples were taken from the jugular vein of three pigs near the average BW in each treatment after 3 hours of fasting on the initial day, week 3, week 6, week 9 and week 12 to measure vitamin E, selenium (Se), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and lymphocytes. All blood samples were collected in serum tubes (SST II Advance; BD Vacutainer, Becton Dickinson, Plymouth, UK) and centrifuged at 1,957 \times g and 4 °C for 15 min (5810R; Eppendorf, centrifuge 5810R, Hamburg, Germany). Subsequently, the supernatant was separated in a microtube (AXYGEN. INC, Union City, CA, USA) and the samples of vitamin E, IL-6 and TNF- α were stored at -20 °C, while the samples of Se and lymphocytes were stored at 4 °C for analysis. Each measurement was conducted using the following analysis machines and techniques: Se (ICP-MS (inductively coupled plasma-mass spectrometry), ICP-MS, Perkin Elmer, Germany), vitamin E (HPLC (high-performance liquid chromatography), HPLC-UVD, PerkinElmer, USA), lymphocytes (flow cytometry, ADVIA 2120, Siemens, Germany), TNF- α (fluorescence, Luminex, Millipore, USA), and IL-6 (fluorescence, Luminex, Millipore, USA).

pH and color of pork

At the end of experiment, four finishing pigs from each treatment were selected and slaughtered for the pork quality analysis. Pork samples were collected from the nearby 10th rib on the right side of carcass. Because of chilling procedure, 30 min after slaughter was regarded as initial time. The pH and pork color were measured at 0, 3, 6, 12 and 24 h, respectively. The pH was measured using a pH meter (Model 720, Thermo, Orion, USA) and pork color was determined by CIE color L*, a*, and b* values using a CM-M6 (Minolta Camera Co., Japan).

Carcass traits and physiochemical properties

The pig carcass grading was judged using the data determined to be grade 5 according to the criterion for grades for pork carcass (scalding) after slaughter at the slaughterhouse. Only results judged to be grades 1+, 1, 2, and 3 were used, and outside grades (grade E) were not included in the grade. The carcass weight was measured by the hot carcass weight of the half carcass. For the back fat thickness, the thickness of the fat layer was measured by cutting the last rib of the carcass, which was pre-cooled to 5 degrees or less at a right angle. Water holding capacity (WHC) was measured by centrifuge method (Ryoichi et al., 1993). Longissimus muscles were ground and sampled in filter tube, then heated in water bath at 80 °C for 20 min and centrifuged for 10 min at 2,000 rpm and 4 °C (Eppendorf centrifuge 5810R, Germany). After that, to calculate the cooking loss, longissimus muscles were packed with polyethylene bag and heated in water bath until the core temperature reached 70 °C and weighed before and after cooking. After heating, the samples were cored (0.5 inch diameter) parallel to muscle fiber and the cores were used to measure the shear force using Warner-Bratzler meat shear machine (Salter 235, GR, USA). Shear force, cooking loss and WHC of pork were analyzed by animal origin food science, Seoul National University.

Pork flavor

For analysis of inosine monophosphate (IMP) which is an indicator to infer the pork flavor, the samples were thawed and centrifuged at 10,000 rpm for 5 min at 4 °C (Eppendorf centrifuge 5417R), and the supernatants were transferred to cold HPLC vials and placed in a thermostated autosampler (1-2 °C). Analysis of IMP was performed by high-performance liquid chromatography (HPLC) (Hewlett-Packard HPLC system series 1100 Germany) using UV detection (210 nm).

Economic benefit

As the experimental pigs were reared in the same environmental condition, economic efficiency was calculated using only the feed cost without considering other factors. The total feed cost and feed cost (won) per body weight gain (kg) were calculated using amount of the total feed intake and feed price. The days to reach market weight (115kg) were estimated from the BW at the end of feeding trial and ADG of 10-11 weeks.

Statistical analysis

All obtained data were first processed by Excel 2010, and then analyzed by one-way ANOVA using Statistical Analysis System® 9.4 TS1M7 (SAS Inst. Inc., Cary, NC, USA). Each pen was used as an experimental unit for growth performance and economic benefit, while individual pigs were used as experimental units for blood profiles, immune response, and pork quality. Orthogonal polynomial contrasts were used to determine the effects of diet (β -glucan and vitamin E against the control), β -glucan, vitamin E, and the interaction between β -glucan and vitamin E. Data are presented as the means and their pooled standard errors. The differences were considered statistically significant when $p < 0.05$, while $0.05 \leq p < 0.10$ was considered to indicate a trend in the data.

RESULTS AND DISCUSSION

Growth performance

The effects of β -glucan with vitamin E supplementation in the growing and finishing pig diet on growth performance are presented in Table 5. As a result of the experiment, ADG was significantly higher in the early finishing period (Diet, $p < 0.01$) and showed higher a trend in the total finishing period (Diet, $p = 0.06$) in the treatments in which β -glucan or vitamin E was added compared to the control. A significant difference in the late finishing period (BG*VE, $p < 0.05$) was found in ADFI by the interaction between β -glucan and vitamin E. The G:F ratio showed significantly higher in the early finishing period (Diet, $p < 0.05$) and a higher trend in the total finishing period (Diet, $p = 0.05$) when β -glucan or vitamin E was added compared to the control. The treatments supplemented with 0.02% vitamin E showed a lower trend in the early growing period (VE, $p = 0.05$) but showed a higher trend in the early finishing period (VE, $p = 0.07$) compared to the treatments without additional vitamin E. In addition, significant difference in the late finishing period (BG*VE, $p < 0.05$) and a trend in the total finishing period (BG*VE, $p = 0.09$) were observed in the G:F ratio by the interaction between β -glucan and vitamin E.

In the previous study by Tran et al. (2021), supplementation with 0.2% β -glucan increased ADG significantly in the growing, finishing, and growing-finishing periods compared to the control when 0.2% β -glucan or 0.4% vitamin E was added to growing and finishing pig feed ($p < 0.05$). In addition, the feed conversion ratio was significantly lower in the treatment where 0.2% β -glucan was added compared to the control ($p < 0.05$). Cueno et al. (2004) studied the effects on growth performance and carcass traits when β -glucan was added to the feed from weaning pigs to growing and finishing pigs. Weaning pigs were assigned to one of four treatments considering sex and initial BW in a 2 x 2 factorial design with two levels of carbadox supplementation (0%/0.25%) and two levels of a product containing β -glucan (0%/0.2%). As a result, the final body weight tended to be higher in the treatments to which 0.2% β -glucan

was added (BG, 0.09). According to Luo et al. (2019), 0.01% β -glucan significantly increased ADG and improved the G:F ratio in 50-75 kg and 25-110 kg sections ($p<0.05$) when β -glucan was added by level (0.005%/0.01%/0.02%) to the growing and finishing pig feed. In addition, supplementation with 0.01% β -glucan significantly increased the ADG and G:F ratio in the 75-110 kg section compared to the control ($p<0.05$). Luo et al. (2019) explained that this was because the digestibility of dry matter, total energy, and crude protein significantly increased with the addition of 0.01% β -glucan, but the exact mechanism for the improvement in growth performance of growing and finishing pigs was not known (Vetvicka et al., 2014). However, β -glucan could have a positive effect on growth performance as a result of improving the intestinal microflora of pigs and promoting the absorption of nutrients in the intestine through improved immunity when β -glucan, an immune enhancer, was added to the feed (Vetvicka et al., 2014). There was no significant difference in the growth performance of growing and finishing pigs when vitamin E was added to the growing and finishing pig feed (Cannon et al., 1996; Wang et al., 2012). However, Hasty et al. (2002) reported that the ADFI of finishing pigs increased linearly when β -glucan was added by level (0.005%/0.01%/0.02%) to the growing and finishing pig feed. Considering previous studies, it is considered that ADG and feed efficiency of the treatment with β -glucan or vitamin E were higher than those of the control because the immunity and health status of the growing and finishing pigs improved.

Consequently, the addition of β -glucan or vitamin E to the growing and finishing pig feed had a positive effect on the growth performance compared to the control.

Blood profiles and immune response

The effects of β -glucan with vitamin E supplementation in the growing and finishing pig diet on blood profiles and immune response are presented in Table 6. The concentration of vitamin E in the blood was significantly higher in the LBE and HBE treatments supplemented with 0.02% vitamin E (VE, $p<0.01$) at the 3rd and 12th weeks and showed higher trends at the 6th and 9th weeks (VE, $p=0.07$; 0.09). In

addition, the concentration of vitamin E in the blood was significantly higher in the treatment where β -glucan and vitamin E were added at the 3rd and 9th weeks compared to the control (Diet, $p < 0.05$), and showed a higher trend at the 6th week (Diet, $p = 0.07$). In addition, the TNF- α concentration showed a lower trend in the treatments to which β -glucan or vitamin E was added compared to the control at week 9 (Diet, $p = 0.07$). Supplementation with 0.05% β -glucan was significantly higher than the treatment with 0.1% β -glucan at 9 and 12 weeks in lymphocyte concentration (Diet, $p < 0.01$). There was no significant difference between treatments in selenium and IL-6 concentrations.

In the present experiment, the lymphocyte concentration was increased when 0.05% β -glucan was added to the feed of growing and finishing pigs. Lymphocytes are part of the adaptive immune system, comprising 20-40% of the white blood cell count (Tigner et al., 2021). Hahn et al. (2006) conducted an experiment by dividing treatments into 4 groups (control, 0.02% β -glucan, antibiotics, and 0.02% β -glucan + antibiotics) to investigate the effect of β -glucan on immunity in weaning pigs. As a result, as a subset of the pig lymphocyte population, major histocompatibility complex-II (week 4), CD-4 and CD-8 (week 8) were found to be higher in the diet supplemented with β -glucan compared to weaning pigs fed other diets. According to Kim et al. (2018), there was no significant difference between treatments on Day 0 before *E. coli* inoculation when β -glucan was added at each level (0.0054%/0.0108%) in weaning pig feed. However, the ratio of CD4+ T cells was significantly increased in the treatment where 0.0054% β -glucan was added after 2 and 5 days of *E. coli* inoculation compared to the control. On the other hand, in the experiments of Lee et al. (2017) and Mao et al. (2005), there was no significant difference in lymphocytes even when β -glucan was added to the weaning pig feed. Regarding the different effects of β -glucan addition on lymphocytes, Bohn and Bemiller (1995) suggested that it may be due to differences in molecular weight, branching degrees, morphology, and intermolecular linkages that can affect the bioactive activity of β -glucan.

In the present experiment, adding 0.02% vitamin E to growing and finishing pig diets increased the concentration of vitamin E in the blood. In particular, these results

are important for growing and finishing pigs. The reason is that the main cause of pork quality deterioration is lipid oxidation, which also increases the occurrence of oxidative rancidity and unpleasant odor. These changes eventually shorten the shelf life of pork. Therefore, antioxidants such as vitamin E are often used to reduce the oxidation of pork and improve shelf life and quality. The results of the present experiment suggest that vitamin E was additionally well delivered to growing and finishing pigs, and it can be expected that it will have a positive effect on the oxidation and quality of pork in finishing pigs in the future.

In conclusion, the addition of 0.05% β -glucan to the growing and finishing pig feed increased the lymphocyte concentration, and the addition of 0.02% vitamin E increased the vitamin E content in the blood.

pH of pork

The effects of β -glucan with vitamin E supplementation in the growing and finishing pig diets on the pH of pork are presented in Table 7. As a result, the pH of pork was significantly higher in the LBE and HBE treatments with the addition of 0.02% vitamin E than in the treatments without vitamin E treatment 3 hours after slaughter (VE, $p < 0.05$). In addition, treatments with β -glucan and vitamin E were significantly lower than the control (Diet, $p < 0.01$). The treatment with β -glucan and vitamin E was significantly higher than the control 24 hours after slaughter (Diet, $p < 0.05$). In addition, there was a significant difference in the pH of pork 24 hours after slaughter due to the interaction of β -glucan and vitamin E (BG*VE, $p = 0.09$).

The change in the pH of pork after slaughter is an important factor in determining the quality of pork and affects the freshness, WHC, softness, color, and storage of the pork (Binder et al., 2004). Additionally, Park et al. (2002) reported that the lower the postmortem pH is, the higher the protein content. Additionally, as the pH increased, cooking loss and drip loss decreased, and the WHC increased. The initial pH and final pH after slaughter are used as standards in judging the quality of pork. The initial pH is the predicted value of PSE meat, and the final pH is the predicted value of DFD.

When blood supply to the muscle is stopped after death, lactic acid production increases due to the anaerobic glycolysis of glycogen stored in the muscle, and the pH of the muscle decreases. This decrease in pH is affected by the handling conditions before and after slaughter, the genetic capacity of the individual, and the rate of anaerobic glycolysis.

The sudden decrease in the pH of pork promotes the outflow of the juice by modifying the protein structure of the muscle, and the outflow of the juice on the surface scatters light to make the pork look pale, resulting in the generation of PSE meat.

According to Luo et al. (2019), the pH of pork was significantly higher and the drip loss could be reduced in the treatment with 0.01% β -glucan than those in the control 45 minutes after slaughter when β -glucan was added by level (0.005%/0.01%/0.02%) in the feed for growing and finishing pigs. According to the experiment of He et al. (2022), the pH of pork was significantly the highest and the drip loss was the lowest 45 minutes after slaughter with a reduced lactic acid content and glycolytic potential in the treatments supplemented with 0.02% β -glucan when β -glucan was added by level (0%/0.005%/0.01%/0.02%/0.04%) in the feed for growing and finishing pigs. This means that the possibility for pork to develop PSE is low. Additionally, no significant difference in the pH of pork was found when β -glucan was added by level in the feed for growing and finishing pigs (Sampah et al., 2021). Moreover, no significant difference in the pH of pork was observed between the treatments when vitamin E was supplemented (Cannon et al., 1996; Wang et al., 2012; Hasty et al., 2002; Li et al., 2015). In the present experiment, since the pH was not out of the normal range (pH 5.3-6.8), there was no negative effect on pork even though it was affected by β -glucan and vitamin E.

In summary, the addition of β -glucan and vitamin E to the feed of growing and finishing pigs does not negatively affect the pH of pigs after slaughter.

Color of pork

The effects of β -glucan with vitamin E supplementation in the growing and

finishing pig diets on the color of pork are presented in Table 8. As a result of the experiment, there was no significant difference in the color of pork between the treatments.

The first thing consumers see when purchasing pork at a store is the color of the meat. Because of these characteristics, the color of the meat has the greatest influence on consumers' understanding of the quality of pork and making a purchase decision. The color of pork is an important indicator for evaluating its muscular appearance. It is influenced by several factors, including the rate of postmortem glycolysis, intramuscular fat, pigment level and the oxidation state of the pigment (Van Oeckel et al., 1999).

According to a study by Luo et al. (2019), the treatment with 0.01% β -glucan 45 minutes after slaughter showed significantly higher redness compared to the control ($p < 0.05$) when β -glucan was added by level (0.005%/0.01%/0.02%) in the feed for growing and finishing pigs. In addition, the yellowness 45 minutes after slaughter was significantly lower than that of the control ($p < 0.05$) in all the treatments where β -glucan was added. According to an experiment by He et al. (2022) when β -glucan was added by level (0%/0.005%/0.01%/0.02%/0.04%) in the feed for finishing pigs, the redness significantly increased (linear, $p < 0.05$), and the whiteness significantly decreased 45 minutes after slaughter (linear, $p < 0.05$). When 0.01% (Cannon et al., 1996), 10 and 210 IU/kg (Wang et al., 2012), and 0.04% (Li et al., 2015) vitamin E were added to growing and finishing pig feed, respectively, there was no significant difference in the color of pork between the treatments.

Bendall and Wismer-pederson (1962) reported that an increase in yellowness and a decrease in the redness of pork were the result of a decrease in the freshness of the pork. Since there is no negative effect on the redness and yellowness of the pork when supplemented with β -glucan and vitamin E, it does not affect the freshness of the pork. In addition, Joo et al. (1995) said that if the L value is 58 or more when 24 hours have elapsed after slaughter, it is PSE pork. However, since all treatments showed an ideal value lower than what Joo et al (1995) suggested, it was determined that there was no negative effect on pork in the present experiment.

As a result, the addition of β -glucan and vitamin E to the growing and finishing pig feed does not have a negative effect on the color of pork.

Carcass traits and physiochemical properties

The effects of β -glucan with vitamin E supplementation in the growing and finishing pig diets on carcass traits and physiochemical properties are presented in Table 9. As a result, there was no significant difference between treatments in carcass traits including carcass weight, carcass yield, grade, and back fat thickness, or in physicochemical properties including cooking loss, shear force, and WHC.

There are standards for pork grading. Carcass weight and back fat thickness are the criteria for the first grade, and appearance, meat quality, and defective items are the secondary grade. In particular, the most important items for grading are carcass weight and back fat thickness, which are the first-grade criteria. Kim and In (2006) analyzed the characteristics of carcass traits by classifying them into 5 levels (less than 69 kg/70~80 kg/81~90 kg/91~96 kg/97 kg or more) according to carcass weight. As a result, back fat thickness increased significantly with increasing carcass weight ($p < 0.05$). In addition, the thickness of back fat was classified into 6 levels (less than 15 mm/16~20 mm/21~25 mm/26~30 mm/31~35 mm/over 36 mm). As a result, the carcass weight became significantly heavier as backfat became thicker ($p < 0.05$).

Cooking loss, which is generally known to have an inverse correlation with WHC, is an indirect indicator of water holding capacity. Shear force is a mechanical measure of the toughness of meat and is known to be highly related to WHC (Hamm, 1986). WHC is a measure of how much meat has moisture according to internal and external environmental changes and is determined by the microstructure of meat or the change in moisture content during shredding. Additionally, it is known to be closely related to changes in the pH of meat. WHC is an important factor in the quality of pork. As the WHC increases, the quality of pork improves, but when the WHC decreases, the shear force gets higher.

According to a study by Luo et al. (2019), there were no significant differences in carcass weight, back fat, and carcass yield among treatments when β -glucan was

added by level (0.005%/0.01%/0.02%) in the feed for growing and finishing pigs. There were no significant differences among the treatments in the cooking loss and shear force. The drip loss was rapidly reduced in the treatment where 0.01% β -glucan was added ($p < 0.05$), which meant that the WHC of the muscles could be improved. Sampath et al. (2021) reported no significant differences in cooking loss and WHC when β -glucan extracted from *Saccharomyces cerevisiae* was added by level (0%/0.05%/0.1%) to the feed for growing and finishing pigs. According to the experiment by He et al. (2022), there were no significant differences in carcass yield and average back fat thickness when β -glucan was added by level (0%/0.005%/0.01%/0.02%/0.04%) in the feed for finishing pigs. When 0.02% β -glucan was added to the treatment, the cooking loss was significantly decreased (linear, $p < 0.05$), but there was no significant difference in shear force. According to Li et al. (2015), when vitamin E (0%/0.04%) and ferulic acid (0%/0.01%) were added to the feed for growing and finishing pigs, there were no significant differences in back fat thickness, lean percentage, and shear force by vitamin E. Cannon et al. (1996) reported that there were no significant differences in carcass yield, back fat thickness, drip loss and cooking loss among treatments when 0.01% vitamin E was added to feed for growing and finishing pigs. Wang et al. (2012) conducted an experiment to add DDGS (0%/15%/30%) and vitamin E (10 and 210 IU/kg) to feed for growing and finishing pigs, but no significant differences were observed in carcass weight, carcass yield, and back fat thickness. In addition, shear force and drip loss were significantly reduced when 210 IU/kg vitamin E was added ($p < 0.05$).

Therefore, the addition of β -glucan and vitamin E to the feed for growing and finishing pigs did not have any effect on carcass characteristics or physicochemical properties.

Pork flavor

The effects of β -glucan with vitamin E supplementation in the growing and finishing pig diets on pork flavor are presented in Table 10. As a result of the experiment, the treatments with β -glucan and vitamin E had a significantly higher

content of IMP than the control (Diet, $p < 0.05$). Additionally, there was a trend in the IMP content ($BG \times VE$, $p < 0.05$) due to the interaction between β -glucan and vitamin E.

IMP is an important indicator to infer the flavor of pork and is particularly related to umami. According to a previous study, supplementing 0.005% and 0.01% β -glucan increased the IMP content in pork when β -glucan was added by level (0.005%/0.01%/0.02%) to the growing and finishing pig feed (Luo et al., 2019). Usually, after slaughter, the oxygen supply to the muscle tissue is stopped, and the energy supplied by phosphocreatine and glycolysis is used for adenosine triphosphate (ATP) synthesis (Zhang et al., 2014). As phosphocreatine and glycolysis decrease, ATP synthesis stops and begins to be degraded, which was thought to increase the contents of IMP in the present experiment.

As a consequence, the addition of β -glucan with vitamin E in growing and finishing pig feed had a positive role in improving the flavor of pork when considering that the content of IMP was increased.

Economic benefit

The effects of β -glucan with vitamin E supplementation in the growing and finishing pig diet on economic benefit are presented in Table 11. In the case of feed cost per weight gain, LBE and HBE treatments with the addition of 0.02% vitamin E showed a high trend in the early growing phase (VE , $p = 0.06$). Supplementing β -glucan or vitamin E showed significantly lower feed cost per weight gain compared to the control in the early finishing phase (Diet, $p < 0.05$). The treatments supplemented with β -glucan or vitamin E showed significantly lower total feed cost compared to the control in the late finishing phase (Diet, $p < 0.05$). No significant difference was found in days to market weight. However, the treatment HBE with 0.1% β -glucan and 0.02% vitamin E showed the shortest days to market weight among treatments.

Tran et al. (2021) conducted an experiment on the effect on economic benefit when 0.2% β -glucan and 0.4% vitamin E were added to the growing and finishing

feed. As a result, the treatment with 0.2% β -glucan had a higher feed price compared to the control. However, the treatment supplemented with β -glucan showed a better effect of 118.98% based on 100% of the control due to the high ADG and low cost of veterinary medicines.

Consequently, since the HBE treatment with 0.1% β -glucan and 0.02% vitamin E had the shortest days to market weight and the lowest total feed cost, a positive effect on economic efficiency can be expected.

CONCLUSION

The ADG and feed efficiency were improved compared to the control when β -glucan or vitamin E was added. Supplementing 0.05% β -glucan significantly increased lymphocyte concentration compared to the addition of 0.1% β -glucan and the content of vitamin E in the blood increased when 0.02% vitamin E was added. The HBE treatment added with 0.1% β -glucan and 0.02% vitamin E showed the most economic effect because it had the shortest days to market weight and the lowest total feed cost. However, carcass traits and meat quality were not affected by β -glucan or vitamin E.

Therefore, the addition of 0.1% β -glucan and 0.02% vitamin E in growing and finishing pig's diet showed great growth performance and economic effects by supplying vitamin E efficiently and by improving the health condition of pigs due to β -glucan.

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Table 1. Formula and chemical compositions of the experimental diets during growing phase 1 (0–3 weeks)

Items	Treatment ¹⁾				
	CON	LB	LBE	HB	HBE
Ingredient (%)					
Ground corn	68.31	68.20	68.16	68.12	68.07
Soybean meal	23.31	23.34	23.34	23.34	23.35
Wheat bran	4.00	4.00	4.00	4.00	4.00
Tallow	1.32	1.35	1.37	1.38	1.40
L-lysine-HCl, 50%	0.07	0.07	0.07	0.07	0.07
DL-methionine, 99%	0.01	0.01	0.01	0.01	0.01
L-threonine, 98.5%	0.01	0.01	0.01	0.01	0.01
L-tryptophan, 20%	0.01	0.01	0.01	0.01	0.01
MDCP	1.44	1.44	1.44	1.44	1.44
Limestone	1.02	1.02	1.02	1.02	1.02
β-glucan ²⁾	0.00	0.05	0.05	0.10	0.10
Vitamin E ²⁾	0.00	0.00	0.02	0.00	0.02
Vitamin Mix ³⁾	0.10	0.10	0.10	0.10	0.10
Mineral Mix ⁴⁾	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Sum	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)⁵⁾					
ME (kcal/kg)	3265.00	3265.00	3265.00	3265.00	3265.00
Crude protein (%)	15.69	15.69	15.69	15.69	15.69
Lysine (%)	0.83	0.83	0.83	0.83	0.83
Methionine (%)	0.25	0.25	0.25	0.25	0.25
Cysteine (%)	0.27	0.27	0.27	0.27	0.27
Threonine (%)	0.60	0.60	0.60	0.60	0.60
Tryptophan (%)	0.15	0.15	0.15	0.15	0.15
Calcium (%)	0.66	0.66	0.66	0.66	0.66
Phosphorus (%)	0.56	0.56	0.56	0.56	0.56

MDCP, mono-dicalcium phosphate; ME, metabolizable energy; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β-glucan; LBE, corn-SBM based diet+0.05% β-glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β-glucan; HBE, corn-SBM based diet+ 0.1% β-glucan+ 0.02% vitamin E

²⁾ β-glucan and vitamin E products were provided by E&T company (E&T Co, Ltd. Daejeon, South Korea).

³⁾ Provided the following quantities of vitamins per kg of complete diet: vitamin A, 16,000 IU; vitamin D₃, 3,200 IU; vitamin E, 35 IU; vitamin K₃, 5mg; riboflavin, 6 mg; calcium pantothenic acid, 16 mg; niacin, 32 mg; D-biotin, 128 ug; vitamin B₁₂, 20 ug

⁴⁾ Provided the following quantities of minerals per kg of complete diet: Fe, 281 mg; Cu, 288 mg; Zn, 143mg; Mn, 49 mg; I, 0.3 mg; Se, 0.3 mg

⁵⁾ Calculated value

Table 2. Formula and chemical compositions of the experimental diets during growing phase 2 (3-6 weeks)

Items	Treatment ¹⁾				
	CON	LB	LBE	HB	HBE
Ingredient (%)					
Ground corn	74.14	74.04	74.00	73.93	73.90
Soybean meal	18.10	18.12	18.12	18.14	18.14
Wheat bran	4.00	4.00	4.00	4.00	4.00
Tallow	0.99	1.02	1.04	1.06	1.07
L-lysine-HCl, 50%	0.01	0.01	0.01	0.01	0.01
DL-methionine, 99%	0.01	0.01	0.01	0.01	0.01
L-threonine, 98.5%	0.01	0.01	0.01	0.01	0.01
L-tryptophan, 20%	0.01	0.01	0.01	0.01	0.01
MDCP	1.30	1.30	1.30	1.30	1.30
Limestone	0.93	0.93	0.93	0.93	0.93
β-glucan ²⁾	0.00	0.05	0.05	0.10	0.10
Vitamin E ²⁾	0.00	0.00	0.02	0.00	0.02
Vitamin Mix ³⁾	0.10	0.10	0.10	0.10	0.10
Mineral Mix ⁴⁾	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Sum	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)⁵⁾					
ME (kcal/kg)	3265.00	3265.00	3265.00	3265.00	3265.00
Crude protein (%)	13.75	13.75	13.75	13.75	13.75
Lysine (%)	0.67	0.67	0.67	0.67	0.67
Methionine (%)	0.23	0.23	0.23	0.23	0.23
Cysteine (%)	0.24	0.24	0.24	0.24	0.24
Threonine (%)	0.52	0.52	0.52	0.52	0.52
Tryptophan (%)	0.15	0.15	0.15	0.15	0.15
Calcium (%)	0.59	0.59	0.59	0.59	0.59
Phosphorus (%)	0.52	0.52	0.52	0.52	0.52

MDCP, mono-dicalcium phosphate; ME, metabolizable energy; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β-glucan; LBE, corn-SBM based diet+0.05% β-glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β-glucan; HBE, corn-SBM based diet+ 0.1% β-glucan+ 0.02% vitamin E

²⁾ β-glucan and vitamin E products were provided by E&T company (E&T Co, Ltd. Daejeon, South Korea).

³⁾ Provided the following quantities of vitamins per kg of complete diet: vitamin A, 16,000 IU; vitamin D₃, 3,200 IU; vitamin E, 35 IU; vitamin K₃, 5mg; riboflavin, 6 mg; calcium pantothenic acid, 16 mg; niacin, 32 mg; D-biotin, 128 ug; vitamin B₁₂, 20 ug

⁴⁾ Provided the following quantities of minerals per kg of complete diet: Fe, 281 mg; Cu, 288 mg; Zn, 143mg; Mn, 49 mg; I, 0.3 mg; Se, 0.3 mg

⁵⁾ Calculated value

Table 3. Formula and chemical compositions of the experimental diets during the finishing phase 1 (6–9 weeks)

Items	Treatment ¹⁾				
	CON	LB	LBE	HB	HBE
Ingredient (%)					
Ground corn	79.31	79.22	79.18	79.12	79.08
Soybean meal	13.33	13.34	13.34	13.35	13.36
Wheat bran	4.00	4.00	4.00	4.00	4.00
Tallow	0.63	0.66	0.68	0.70	0.71
L-lysine-HCl, 50%	0.21	0.21	0.21	0.21	0.21
DL-methionine, 99%	0.01	0.01	0.01	0.01	0.01
L-threonine, 98.5%	0.01	0.01	0.01	0.01	0.01
L-tryptophan, 20%	0.01	0.01	0.01	0.01	0.01
MDCP	1.15	1.15	1.15	1.15	1.15
Limestone	0.84	0.84	0.84	0.84	0.84
β-glucan ²⁾	0.00	0.05	0.05	0.10	0.10
Vitamin E ²⁾	0.00	0.00	0.02	0.00	0.02
Vitamin Mix ³⁾	0.10	0.10	0.10	0.10	0.10
Mineral Mix ⁴⁾	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Sum	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)⁵⁾					
ME (kcal/kg)	3265.00	3265.00	3265.00	3265.00	3265.00
Crude protein (%)	12.13	12.13	12.13	12.13	12.13
Lysine (%)	0.66	0.66	0.66	0.66	0.66
Methionine (%)	0.21	0.21	0.21	0.21	0.21
Cysteine (%)	0.21	0.21	0.21	0.21	0.21
Threonine (%)	0.46	0.46	0.46	0.46	0.46
Tryptophan (%)	0.12	0.12	0.12	0.12	0.12
Calcium (%)	0.52	0.52	0.52	0.52	0.52
Phosphorus (%)	0.47	0.47	0.47	0.47	0.47

MDCP, mono-dicalcium phosphate; ME, metabolizable energy; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β-glucan; LBE, corn-SBM based diet+0.05% β-glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β-glucan; HBE, corn-SBM based diet+ 0.1% β-glucan+ 0.02% vitamin E

²⁾ β-glucan and vitamin E products were provided by E&T company (E&T Co, Ltd. Daejeon, South Korea).

³⁾ Provided the following quantities of vitamins per kg of complete diet: vitamin A, 16,000 IU; vitamin D₃, 3,200 IU; vitamin E, 35 IU; vitamin K₃, 5mg; riboflavin, 6 mg; calcium pantothenic acid, 16 mg; niacin, 32 mg; D-biotin, 128 ug; vitamin B₁₂, 20 ug

⁴⁾ Provided the following quantities of minerals per kg of complete diet: Fe, 281 mg; Cu, 288 mg; Zn, 143mg; Mn, 49 mg; I, 0.3 mg; Se, 0.3 mg

⁵⁾ Calculated value

Table 4. Formula and chemical compositions of the experimental diets during finishing phase 2 (9–12 weeks)

Items	Treatment ¹⁾				
	CON	LB	LBE	HB	HBE
Ingredient (%)					
Ground corn	84.41	84.30	84.27	84.20	84.17
Soybean meal	8.73	8.75	8.75	8.77	8.77
Wheat bran	4.00	4.00	4.00	4.00	4.00
Tallow	0.35	0.39	0.40	0.42	0.43
L-lysine-HCl, 50%	0.17	0.17	0.17	0.17	0.17
DL-methionine, 99%	0.01	0.01	0.01	0.01	0.01
L-threonine, 98.5%	0.01	0.01	0.01	0.01	0.01
L-tryptophan, 20%	0.02	0.02	0.02	0.02	0.02
MDCP	1.05	1.05	1.05	1.05	1.05
Limestone	0.75	0.75	0.75	0.75	0.75
β-glucan ²⁾	0.00	0.05	0.05	0.10	0.10
Vitamin E ²⁾	0.00	0.00	0.02	0.00	0.02
Vitamin Mix ³⁾	0.10	0.10	0.10	0.10	0.10
Mineral Mix ⁴⁾	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Sum	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)⁵⁾					
ME (kcal/kg)	3265.00	3265.00	3265.00	3265.00	3265.00
Crude protein (%)	10.43	10.43	10.43	10.43	10.43
Lysine (%)	0.52	0.52	0.52	0.52	0.52
Methionine (%)	0.19	0.19	0.19	0.19	0.19
Cysteine (%)	0.20	0.20	0.20	0.20	0.20
Threonine (%)	0.38	0.38	0.38	0.38	0.38
Tryptophan (%)	0.10	0.10	0.10	0.10	0.10
Calcium (%)	0.46	0.46	0.46	0.46	0.46
Phosphorus (%)	0.43	0.43	0.43	0.43	0.43

MDCP, mono-dicalcium phosphate; ME, metabolizable energy; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β-glucan; LBE, corn-SBM based diet+0.05% β-glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β-glucan; HBE, corn-SBM based diet+ 0.1% β-glucan+ 0.02% vitamin E

²⁾ β-glucan and vitamin E products were provided by E&T company (E&T Co, Ltd. Daejeon, South Korea).

³⁾ Provided the following quantities of vitamins per kg of complete diet: vitamin A, 16,000 IU; vitamin D₃, 3,200 IU; vitamin E, 35 IU; vitamin K₃, 5mg; riboflavin, 6 mg; calcium pantothenic acid, 16 mg; niacin, 32 mg; D-biotin, 128 ug; vitamin B₁₂, 20 ug

⁴⁾ Provided the following quantities of minerals per kg of complete diet: Fe, 281 mg; Cu, 288 mg; Zn, 143mg; Mn, 49 mg; I, 0.3 mg; Se, 0.3 mg

⁵⁾ Calculated value

Table 5. Effects of β -glucan with vitamin E supplementation on growth performance in growing and finishing pigs

Items	Treatment ¹⁾					SEM	P-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Body weight, kg										
Initial	34.41	34.43	34.43	34.42	34.45	0.013	-	-	-	-
Week 3	47.76	48.00	47.00	47.75	47.25	0.544	0.87	0.99	0.59	0.86
Week 6	67.85	65.80	65.45	66.34	64.01	0.779	0.25	0.81	0.47	0.60
Week 9	88.45	89.29	89.32	89.35	88.66	0.541	0.64	0.82	0.81	0.79
Week 12	108.41	108.99	108.68	108.56	109.49	0.384	0.63	0.84	0.74	0.52
Average daily gain, g										
0-3 weeks	635.75	645.88	598.50	634.98	609.57	19.129	0.80	0.99	0.45	0.82
3-6 weeks	956.46	847.62	878.06	885.04	797.96	26.160	0.13	0.72	0.64	0.33
0-6 weeks	796.10	746.93	738.28	760.00	703.77	17.907	0.23	0.80	0.45	0.58
6-9 weeks	981.05	1118.54	1137.42	1095.41	1173.47	24.412	0.01	0.90	0.33	0.55
9-12 weeks	950.30	937.94	921.43	914.71	992.44	17.232	0.85	0.56	0.46	0.26
6-12 weeks	965.67	1028.24	1029.42	1005.06	1082.95	14.931	0.06	0.63	0.21	0.23
0-12 weeks	880.89	887.59	883.85	882.54	893.36	5.228	0.69	0.87	0.79	0.58
Average daily feed intake, g										
0-3 weeks	1456.97	1445.41	1544.22	1345.07	1513.95	33.946	0.95	0.40	0.10	0.65
3-6 weeks	2435.89	2412.08	2236.40	2176.36	2229.08	56.248	0.25	0.36	0.64	0.39
0-6 weeks	1946.43	1928.74	1890.31	1760.72	1871.51	38.031	0.41	0.31	0.69	0.41
6-9 weeks	3115.82	3004.25	2768.71	3020.07	2864.12	54.359	0.14	0.64	0.11	0.74
9-12 weeks	3361.35	2861.31	3221.22	3561.14	3061.98	84.944	0.32	0.12	0.67	0.02
6-12 weeks	3225.66	2940.32	2971.15	3262.13	2952.63	56.191	0.15	0.21	0.24	0.16
0-12 weeks	2432.44	2294.52	2289.24	2356.08	2271.47	36.659	0.20	0.80	0.61	0.65
Gain : Feed ratio (G:F ratio)										
0-3 weeks	0.436	0.450	0.387	0.476	0.404	0.0145	0.85	0.50	0.05	0.88
3-6 weeks	0.398	0.355	0.395	0.410	0.363	0.0152	0.71	0.78	0.96	0.27
0-6 weeks	0.413	0.390	0.393	0.433	0.378	0.0119	0.69	0.62	0.35	0.32
6-9 weeks	0.316	0.376	0.412	0.363	0.414	0.0121	0.01	0.81	0.07	0.75
9-12 weeks	0.286	0.331	0.290	0.257	0.326	0.0104	0.54	0.38	0.51	0.02
6-12 weeks	0.301	0.353	0.348	0.309	0.369	0.0094	0.05	0.53	0.15	0.09
0-12 weeks	0.363	0.390	0.388	0.375	0.395	0.0061	0.15	0.79	0.52	0.44

SEM, standard error of mean; BG, β -glucan; VE, vitamin E; SBM, soymeal meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β -glucan; LBE, corn-SBM based diet+0.05% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β -glucan; HBE, corn-SBM based diet+ 0.1% β -glucan+ 0.02% vitamin E

Table 6. Effects of β -glucan with vitamin E supplementation on blood profiles and immune response in growing and finishing pigs

Items	Treatment ¹⁾					SEM	P-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Vitamin E ($\mu\text{mol/L}$)										
Initial		-----2.23 -----				-	-	-	-	-
Week 3	2.65	2.68	5.53	3.63	5.75	0.396	0.02	0.36	<0.01	0.57
Week 6	3.58	4.85	7.63	4.68	5.88	0.510	0.07	0.36	0.07	0.45
Week 9	4.75	6.45	8.98	6.95	7.93	0.506	0.02	0.78	0.09	0.44
Week 12	5.70	6.85	9.48	5.23	7.58	0.553	0.21	0.13	0.04	0.90
Selenium ($\mu\text{g/L}$)										
Initial		----- 167.34 -----				-	-	-	-	-
Week 3	182.00	172.75	175.00	177.00	177.75	2.234	0.30	0.52	0.78	0.89
Week 6	190.75	202.00	186.00	174.75	186.00	3.743	0.69	0.11	0.77	0.11
Week 9	207.50	206.00	187.25	178.25	201.00	5.087	0.26	0.53	0.86	0.08
Week 12	206.75	212.75	209.00	203.50	197.25	5.524	0.94	0.45	0.72	0.93
TNF-α ($\mu\text{g/mL}$)										
Initial		----- 0.07 -----				-	-	-	-	-
Week 3	0.09	0.42	0.06	0.02	0.02	0.058	0.76	0.09	0.15	0.15
Week 6	0.13	0.56	0.02	0.10	0.10	0.076	0.70	0.12	0.28	0.10
Week 9	0.46	0.26	0.12	0.07	0.03	0.071	0.07	0.57	0.36	0.75
Week 12	0.34	0.02	0.18	1.05	0.06	0.151	0.99	0.16	0.20	0.09
IL-6 ($\mu\text{g/mL}$)										
Initial		----- 0.13 -----				-	-	-	-	-
Week 3	0.11	1.18	0.22	0.02	0.04	0.197	0.58	0.16	0.25	0.31
Week 6	0.24	2.34	0.02	0.10	0.34	0.268	0.99	0.50	0.52	0.27
Week 9	1.22	2.66	0.41	0.07	0.32	0.301	0.10	0.64	0.70	0.88
Week 12	0.96	0.45	0.63	1.05	1.55	0.416	0.73	0.11	0.62	0.49
Lymphocyte (%)										
Initial		----- 52.53 -----				-	-	-	-	-
Week 3	53.95	61.93	57.00	51.55	50.90	2.859	0.86	0.24	0.69	0.76
Week 6	49.98	56.48	56.02	47.20	52.33	2.151	0.60	0.22	0.65	0.59
Week 9	59.30	64.05	60.15	51.00	51.40	1.750	0.48	<0.01	0.60	0.52
Week 12	60.45	64.03	67.37	56.08	57.93	1.249	0.70	<0.01	0.23	0.72

SEM, standard error of mean; BG, β -glucan; VE, vitamin E, TNF- α , tumor necrosis factor- α ; IL, interleukin;

SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β -glucan; LBE, corn-SBM based diet+0.05% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β -glucan; HBE, corn-SBM based diet+ 0.1% β -glucan+ 0.02% vitamin E

Table 7. Effects of β -glucan with vitamin E supplementation on pH in growing and finishing pigs

Items	Treatment ¹⁾					SEM	P-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Time after slaughter										
0 hour	5.75	5.74	5.62	5.65	5.68	0.026	0.27	0.78	0.48	0.22
3 hours	5.72	5.49	5.64	5.43	5.52	0.031	<0.01	0.11	0.03	0.58
6 hours	5.36	5.44	5.55	5.46	5.46	0.030	0.18	0.63	0.44	0.47
12 hours	5.54	5.46	5.58	5.51	5.52	0.022	0.74	0.90	0.23	0.31
24 hours	5.32	5.41	5.49	5.44	5.34	0.020	0.03	0.14	0.71	0.03

SEM, standard error of mean; BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β -glucan; LBE, corn-SBM based diet+0.05% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β -glucan; HBE, corn-SBM based diet+ 0.1% β -glucan+ 0.02% vitamin E

Table 8. Effects of β -glucan with vitamin E supplementation on pork color in growing and finishing pigs

Items	Treatment ¹⁾					SEM	P-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
CIE value²⁾, L*										
0 hour	49.76	47.30	43.87	36.70	43.50	1.618	0.14	0.28	0.96	0.32
3 hours	41.87	46.79	45.58	44.87	49.09	1.182	0.13	0.77	0.58	0.32
6 hours	45.20	47.32	42.72	39.04	41.77	1.620	0.56	0.23	0.80	0.34
12 hours	44.16	47.84	42.37	46.16	44.16	1.068	0.73	0.98	0.15	0.49
24 hours	44.27	53.07	50.06	46.82	41.45	1.709	0.39	0.06	0.26	0.75
CIE value²⁾, a*										
0 hour	2.50	2.97	3.52	3.19	3.57	0.141	0.12	0.64	0.12	0.75
3 hours	2.74	3.11	2.60	3.46	2.70	0.209	0.68	0.65	0.21	0.80
6 hours	3.35	2.42	3.45	2.95	3.44	0.207	0.60	0.58	0.12	0.57
12 hours	2.89	3.35	2.54	2.65	3.77	0.259	0.78	0.66	0.80	0.13
24 hours	3.58	1.97	2.22	2.58	4.00	0.361	0.33	0.15	0.31	0.46
CIE value²⁾, b*										
0 hour	8.88	8.80	8.76	7.86	9.30	0.445	0.87	0.86	0.53	0.51
3 hours	10.04	9.18	9.19	8.87	8.04	0.346	0.19	0.37	0.61	0.60
6 hours	10.80	9.54	9.78	9.41	9.08	0.389	0.21	0.67	0.96	0.76
12 hours	11.09	9.41	10.23	8.54	9.66	0.396	0.12	0.42	0.28	0.86
24 hours	8.55	8.75	8.83	9.34	9.51	0.335	0.55	0.45	0.88	0.96

SEM, standard error of mean; BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β -glucan; LBE, corn-SBM based diet+0.05% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β -glucan; HBE, corn-SBM based diet+ 0.1% β -glucan+ 0.02% vitamin E

²⁾ CIE L* : luminance or brightness (vary from black to white)

CIE a* : red-green component (+a=red, -a=green)

CIE b* : yellow-blue component (+b=yellow, -b=blue)

Table 9. Effects of β -glucan with vitamin E supplementation on carcass traits and physiochemical properties in growing and finishing pigs

Items	Treatment ¹⁾					SEM	P-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Carcass traits										
Carcass weight, kg	85.25	86.50	88.00	84.00	86.00	0.478	-	-	-	-
Carcass yield, %	77.33	77.06	77.36	76.71	76.96	0.090	-	-	-	-
Grade ²⁾	1.50	2.25	1.50	1.25	1.50	0.168	0.77	0.20	0.52	0.20
Backfat thickness, mm	23.50	24.50	24.00	21.75	22.75	0.952	0.92	0.40	0.92	0.75
Physiochemical properties										
Cooking loss ³⁾	20.52	22.70	21.40	22.63	19.36	0.463	0.33	0.26	0.12	0.29
Shear force ⁴⁾	30.13	38.25	30.16	35.20	31.98	2.007	0.49	0.90	0.25	0.61
WHC	72.15	68.97	68.54	67.29	70.65	0.984	0.22	0.93	0.53	0.42

SEM, standard error of mean; BG, β -glucan; VE, vitamin E; WHC, water holding capacity; SBM, soybean meal;

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β -glucan; LBE, corn-SBM based diet+0.05% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β -glucan; HBE, corn-SBM based diet+ 0.1% β -glucan+ 0.02% vitamin E

²⁾ Grade: grade 1+ =1; grade 1 =2; grade 2 =3

³⁾ Cooking loss unit: %

⁴⁾ Shear force unit: kg/0.5 inch²

Table 10. Effects of β -glucan with vitamin E supplementation on inosine monophosphate (IMP) in growing and finishing pigs

Items	Treatment ¹⁾					SEM	P-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
IMP (mg/kg)	1,097.5	1,420.8	1,787.1	1,947.8	1,411.8	103.02	0.02	0.70	0.67	0.03

SEM, standard error of mean; BG, β -glucan; VE, vitamin E; IMP, inosine monophosphate; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β -glucan; LBE, corn-SBM based diet+0.05% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β -glucan; HBE, corn-SBM based diet+ 0.1% β -glucan+ 0.02% vitamin E

Table 11. Effects of β -glucan with vitamin E supplementation on economic benefit in growing and finishing pigs

Items	Treatment ¹⁾					SEM	P-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Feed cost per weight gain, won/kg										
0-3 weeks	1,199	1,174	1,351	1,131	1,305	39.2	0.31	0.79	0.06	0.99
3-6 weeks	1,349	1,529	1,333	1,290	1,468	57.3	0.70	0.86	0.95	0.18
6-9 weeks	1,610	1,349	1,216	1,384	1,231	47.2	0.02	0.10	0.12	0.91
9-12 weeks	1,699	1,585	1,625	1,803	1,486	47.1	0.33	0.92	0.19	0.10
0-12 weeks	961	900	902	929	888	13.2	0.26	0.45	0.52	0.48
Total feed cost per pig, won/head										
0-3 weeks	15,910	15,783	16,862	14,688	16,523	370.7	0.39	0.57	0.10	0.65
3-6 weeks	26,600	26,339	24,421	23,765	24,341	614.2	0.52	0.44	0.63	0.38
6-9 weeks	32,716	31,544	29,071	31,710	30,073	570.7	0.26	0.70	0.11	0.74
9-12 weeks	38,869	30,873	31,197	34,252	31,002	589.9	0.04	0.31	0.24	0.16
0-12 weeks	104,205	98,297	98,071	100,934	97,309	1570.4	0.46	0.59	0.61	0.65
Days to market weight (reached at 115 kg body weight), days										
	107.7	107.5	106.7	105.3	104.0	0.58	0.21	0.11	0.42	0.84

SEM, standard error of mean; BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON, corn-SBM based diet; LB, corn-SBM based diet+0.05% β -glucan; LBE, corn-SBM based diet+0.05% β -glucan+0.02% vitamin E; HB, corn-SBM based diet+0.1% β -glucan; HBE, corn-SBM based diet+ 0.1% β -glucan+ 0.02% vitamin E

Chapter V. Effects of β -glucan with vitamin E supplementation on the physiological response, litter performance, blood profiles, immune response, and milk composition of lactating sows

ABSTRACT: This study was conducted to evaluate the effects of β -glucan and vitamin E diet supplementation on the physiological response, litter performance, blood profiles, immune response, and milk composition of lactating sows. A total of 50 multiparous F₁ sows (Yorkshire \times Landrace) with an average body weight (BW) of 233.6 ± 4.30 kg and an average parity of 4.00 ± 0.307 and their litters were used in this experiment. All sows were allotted one of five treatments, taking into consideration BW, backfat thickness, and parity in a completely randomized design with 10 replicates. The experimental diets included a corn–soybean meal-based basal diet with or without 0.1% or 0.2% β -glucan and 110 IU vitamin E/kg diet. All treatments added with β -glucan or vitamin were statistically higher in the average daily feed intake (ADFI) measurement of lactating sows compared to those of the control (Diet, $p < 0.01$). Additionally, the ADFI of lactating sows was significantly higher in the groups supplemented with 0.1% β -glucan compared to 0.2% β -glucan (BG, $p < 0.01$). There was an increasing trend in piglet weight at weaning (BG, $p = 0.07$), litter weight at the 21st day of lactation (BG, $p = 0.07$) and litter weight gain (BG, $p = 0.08$) in groups supplemented with 0.1% β -glucan. The addition of 110 IU vitamin E/kg diet increased vitamin E concentration significantly in lactating sows (VE, $p < 0.01$) and exhibited a trend for higher concentrations of vitamin E (VE, $p = 0.09$) in piglets. Adding 0.1% β -glucan compared to 0.2% β -glucan showed a lower trend in TNF- α concentration in lactating sows (BG, $p = 0.06$) and in piglets (BG, $p = 0.09$) on the 21st day of lactation. There were no significant differences in the milk composition of sows. Consequently, adding 0.1% β -glucan and 110 IU vitamin E/kg to a lactating sow's diet was beneficial to the growth performance of piglets by leading to an increase in the feed intake of sows and efficiently supplying vitamin E to both the sows and piglets.

Key words: β -glucan; Vitamin E; Lactating Sow; Piglet; Piglet Growth Performance

INTRODUCTION

Much effort has been made to develop feed that meets the increase in litter size and milk production of improved sows. In addition, milk demand by piglets increased as the number of litters increased, but the production of sow's milk has been insufficient (Kim et al., 2005). Therefore, proper nutritional management to improve sow productivity is a major issue in the swine industry. β -glucan is one of many feed additives used for improving the performance of piglets through the nutritional management of sows and for increasing the milk production and productivity of sows (Zhan et al., 2011).

Oxidative stress can negatively affect the fertility of animals and animal welfare in animals with genetic improvement. Oxidative stress is known to cause fetal growth disorders, infertility, and stillbirth, especially during gestation, and is involved in the occurrence of various complications by damaging deoxyribonucleic acid in the sow's body and destroying normal cells (Prater et al., 2008). Therefore, the defense mechanism of antioxidants such as vitamin E against oxidative stress is important (Surai et al., 2016).

β -glucan is a type of functional polysaccharide produced by bacteria, yeast, fungi, or cereals. It has various biological functions, such as improving immunity, preventing disease, and controlling blood glucose levels. According to previous studies, β -glucan is known to improve growth performance when added to swine feed (Lee et al., 2017). The addition of β -glucan to swine diets can control the synthesis of the inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) and regulate the metabolism of nutrients that negatively affect growth performance (Li et al., 2006). In addition, it can increase phagocytosis and the production of IL-2 and decrease the concentrations of cortisol and TNF- α in the blood (Vetvicka and Oliveira, 2014).

Vitamin E is a fat-soluble substance that exists in eight compound forms (α -, β -, γ - and δ -tocopherols and tocotrienols) that function as antioxidants in plant and animal tissues. α -tocopherol is known to have the highest physiological activity

among isomers of vitamin E in animal bodies (Traber, 2007). Vitamin E acts as an antioxidant at the cell membrane level, and its deficiency can cause the degeneration of skeletal and cardiac muscle, gastric ulcer formation, and anemia. Vitamin E performs several functions related to reproduction. The amount of maternal vitamin E transferred through the placenta during gestation is very small, and newborn piglets must obtain their daily needs from colostrum or milk. When vitamin E was added to feed of lactating sows, the level of vitamin E in the milk of sows was increased to prevent vitamin E deficiency in piglets and improve the health of sows (Mahan, 1991; Mahan, 1994; Shelton et al., 2014).

As described above, there have been many studies describing the effects and advantages of β -glucan or vitamin E diet supplementation. However, there have been no experiments on the effects of supplementing diets with β -glucan and vitamin E together on reproductive performance, litter performance, blood profiles, immune response or milk composition in lactating sows. Therefore, this study was conducted to evaluate the effects of β -glucan and vitamin E diet supplementation on the physiological responses, litter performance, blood profiles, immune response and milk composition of lactating sows.

MATERIALS AND METHODS

All experimental procedures involving animals were conducted in accordance with the Animal Experimental Guidelines provided by the Seoul National University Institutional Animal Care and Use Committee (SNUIACUC; SNU-200209-2)

Experimental animals and housing environment

A total of 50 multiparous F₁ sows (Yorkshire × Landrace) with an average body weight (BW) of 233.6 ± 4.30 kg and an average parity of 4.00 ± 0.307 and their litters were used in this experiment. All sows were allotted to one of five treatments taking into consideration BW, backfat thickness, and parity in a completely randomized design (CRD) with 10 replicates. During the lactating period, the animals were raised in a farrowing frame (2.5×1.8 m²), and the temperature and ventilation of the farrowing house were automatically controlled by a ventilation fan and an automatic control device. Piglets were cut off the umbilical cord and had their tails cut 3 days postpartum, and male piglets were castrated. Additionally, iron preparations (150 mg/ml) were also injected once.

Experimental design and diet

The dietary treatments included 1) CON (corn–soybean meal (SBM)-based diet), 2) LB (corn-SBM-based diet + 0.1% β-glucan), 3) LBE (corn-SBM-based diet + 0.1% β-glucan + 110 IU vitamin E/kg), 4) HB (corn-SBM-based diet + 0.2% β-glucan), and 5) HBE (corn-SBM-based diet + 0.2% β-glucan + 110 IU vitamin E/kg). A corn-SBM-based diet was used as feed in this experiment, and all nutrients in this experimental diet met or exceeded the nutrient requirements of the National Research Council (NRC) 2012 on lactating sows. Choline was not added to the premix, and 0.1% choline-chloride was fed in the same way as the experimental feed. In the present study, β-glucan and vitamin E products were provided by E&T company (E&T CO., Ltd, Daejeon, South Korea). β-glucan product consists of cell walls of

brewer's yeast (100% *Saccharomyces cerevisiae*), rich in (1,3)-(1,6)-B-D-glucans and mannans. It is fine solid powder with beige to light brown color. Vitamin E was in the form of vitamin E-acetate. In the case of vitamin E, 68 IU/kg was present in the vitamin premix, and 110 IU/kg of vitamin E was additionally supplemented to the LBE and HBE treatments. All nutrient contents in the feed were formulated equally, and the formula and chemical composition of the experimental diet are presented in Table 1. The crude protein content in the lactating sow feed was 13.43%, the lysine content was 0.96%, the methionine content was 0.29%, the calcium content was 0.90%, and the total phosphorus content was 0.70%.

Physiological response

The live body weight and backfat thickness of sows were measured at 24 hours postpartum and on Day 21 of lactation. The body weight of the sow was measured by an electric scale (CAS Co. Ltd., Yangju-si, Gyeonggi-do, Korea), and backfat thickness was measured at the P2 position (the mean value from both sides of the last rib and 65 mm away from the backbone) by an ultrasound device (Lean Meter®, Renco Corp., Minneapolis, MN, USA). Daily feed waste was recorded during lactation to identify the real feed intake of each sow during the experimental period. The weaning-to-estrus interval (WEI) of sows was monitored after weaning as an important parameter for evaluating reproductive performance.

Litter performance

The BW and average daily gain (ADG) of piglets were measured within 24 hours postpartum and after 21 days of lactation to determine the growth performance of piglets and the lactating ability of sows with initiation of lactation after cross fostering.

Blood profiles and immune response

Blood samples (n=5 for each treatment) were collected from the jugular vein of sows using 10 ml disposable syringes at 24 hours postpartum and after 21 days of

lactation. Additionally, blood samples (n=5 for each treatment) were collected from the jugular vein of piglets using 3 ml disposable syringes at 24 hours postpartum and 5 ml disposable syringes at 21 days of lactation. All blood samples were collected in serum tubes (SSTTM II Advance, BD Vacutainer, Becton Dickinson, Plymouth, UK) and centrifuged at $1,957 \times g$ and 4 °C for 15 min (5810R, Eppendorf, Hamburg, centrifuge 5810R, Germany). Subsequently, the supernatant was separated in a microtube (AXYGEN. INC, Union City, CA, USA) and the samples of vitamin E, IL-6 and TNF- α were stored at -20 °C, while the samples of selenium and lymphocytes were stored at 4 °C for analysis. Each measurement was conducted using the following analysis machines and techniques: Se (ICP-MS (Inductively coupled plasma-mass spectrometry), ICP-MS, Perkin Elmer, Germany), vitamin E (HPLC (High-performance liquid chromatography), HPLC-UVD, PerkinElmer, USA), lymphocytes (Flow cytometry, ADVIA 2120, Siemens, Germany), TNF- α (Fluorescent, Luminex, Millipore, USA), and IL-6 (Fluorescent, Luminex, Millipore, USA).

Milk composition

Colostrum samples (n=4 for each treatment) were collected from functional mammary glands at 24 hours postpartum, and milk samples (n=4 for each treatment) were taken at 21 days of lactation. Colostrum and milk were collected from the first and second teats by injecting 10 IU/mL oxytocin (Komi oxytocin inj., Komipharm International Co. Ltd., Siheung-si, Gyeonggi-do, Korea) into the blood vessel of the sows' ear and stored in 50 mL conical tubes (SPL Life Sciences Co., Ltd., Pocheon-si, Gyeonggi-do, Korea). The collected samples were stored in a freezer (-20 °C) until further analysis. Proximate analyses for casein, fat, protein, lactose, total solid and solid not fat (SNF) compositions of milk as well as colostrum were determined using a MilkoScan FT 120 (FOSS, Hillerod, Denmark).

Statistical analysis

All obtained data were processed by Excel 2010 first, and then analyzed by one-way ANOVA procedure using Statistical Analysis System® 9.4 TS1M7 (SAS Inst. Inc., Cary, NC, USA). Individual sows and their litters were used as the experimental unit in the physiological response, litter performance, blood profiles, immune response, and milk composition. The orthogonal polynomial contrasts were used to determine the effects of diet (β -glucan and vitamin E against the control), β -glucan, vitamin E, as well as the interaction between β -glucan and vitamin E. Data were presented as means and their pooled standard errors. The differences were considered as statistically significant when $p < 0.05$, while $0.05 \leq p < 0.10$ was considered to indicate a trend in the data.

RESULTS AND DISCUSSION

Physiological response

The effects of β -glucan with vitamin E supplementation in the lactating sow diet on BW, backfat thickness, average daily feed intake (ADFI) and WEI during lactation are shown in Table 2. There were no significant differences in BW and backfat thickness of sows at 24 hours postpartum or on day 21 of lactation. Moreover, WEI was not affected by β -glucan and vitamin E. However, the ADFI of lactating sows were statistically higher in all treatments added with β -glucan or vitamin E than in the control (Diet, $p < 0.01$). Additionally, the ADFI of lactating sows was significantly higher in the treatments with 0.1% β -glucan than in the treatments with 0.2% β -glucan (BG, $p < 0.01$).

Szuba-Trznadel et al. (2014) conducted an experiment by adding different β -glucan percentages (0%, 0.01%, 0.02%, and 0.03%) in sow feed. β -glucan had no effect on the BW and backfat thickness of sows between treatments. In addition, no significant differences were found in BW and backfat thickness of sows between treatments according to a different study by Shelton et al. (2014), which was conducted with the addition of varying concentrations of vitamin E (15, 30, 45, 60 IU D- α -TAc and 44, 66 IU DL- α -TAc). In most previous studies, as in the results of the current experiment, there was no statistically significant difference in BW and backfat thickness of sows when β -glucan and vitamin E were supplemented in the sow feed (Mahan, 1994; Veum et al., 1995; Mahan et al., 2000). In the present experiment, when β -glucan and vitamin E were added to the feed of lactating sows, the feed intake of lactating sows was significantly increased. Therefore, we concluded that there was no negative effect on BW and backfat thickness, as there was no BW and backfat thickness loss in sows after consuming sufficient nutrients for pig milk production.

In general, it is known that the nutritional and metabolic status of the sows during the lactation period affected their WEI (Pettigrew, 1998). When the ADFI of lactating sows was low, the postweaning health status of lactating sows became poor, which

led to an increase in the WEI (Reese et al., 1982). Additionally, it is known that WEI is affected by the lactation period, weight loss during lactation, and the number of piglets (Eissen et al., 2000). Since there were no significant differences in BW, backfat thickness, or the number of piglets, we concluded that no significant difference was observed even in the WEI.

The feed intake of lactating sows had a strong relationship with changes in weight and backfat thickness. If sufficient nutrients for milk production were not consumed by sows, the nutrients accumulated in the body during gestation were used for milk production, which may negatively affect subsequent reproductive performance (Reese et al., 1982). Shen et al. (2019) conducted an experiment comparing sow feed with 15 g/d topdressing of β -glucan during the lactation period with the control group and reported that there was no significant difference in ADFI during the experimental period. In an experiment by Kim et al. (2008), no significant difference was found in the ADFI of primiparous and multiparous sows compared to the control when the sows were fed a diet top-dressed with 15 g/d β -glucan.

However, in the current experiment, all treatments with β -glucan supplementation exhibited significantly higher ADFI than the control ($p < 0.01$). The mechanism for the improvement of growth performance after the addition of β -glucan in swine feed is not precisely known (Vetvicka and Oliveira, 2014; Luo et al., 2019). However, β -glucan has been shown to improve growth performance by promoting gut health and body immunity in several other animal studies, including mice, chickens, and cattle (Luo et al., 2019). In addition, β -glucan is a type of natural immune enhancer that increases the mucosal function of the intestinal wall and improves the gastrointestinal environment. This contributed to the growth of animals such as pigs by aiding the digestion of nutrients in the stomach and improving absorption into the body through the intestinal mucosa (Luo et al., 2019; Sørensen, 2005). Regarding the studies mentioned above, we considered that the ADFI was higher in the experimental groups than the control group because the immunity and health status of the lactating sows improved. In addition, the treatments supplemented with 0.1% β -glucan exhibited significantly higher ADFI than the treatments supplemented with 0.2% β -glucan (BG,

p<0.01). Szuba-Trznadel et al. (2014) conducted an experiment by adding varying levels of β -glucan extracted from yeast to the feed of lactating sows (0%, 0.01%, 0.02%, and 0.03%). The ADFI increased linearly from 0% to 0.02% β -glucan treatments, but decreased at 0.03%. Li et al. (2006) supplemented the feed of weaning pigs with varying levels of β -glucan (0%, 0.0025%, 0.005%, 0.01%, and 0.02%) and observed that ADFI increased from 0% to 0.005% β -glucan supplemented groups but decreased in the 0.01% group. As in the results of the previous studies described above, various results have been obtained depending on the level of β -glucan added. Differences in the purity, form, and physical state of β -glucan may have influenced these results (Li et al., 2006). Additionally, it was thought that various amounts of β -glucan can induce or inhibit the expression of inflammatory cytokines, thus promoting the secretion of proinflammatory cytokines and decreasing the secretion of anti-inflammatory cytokines, which may have affected feed intake.

In conclusion, when β -glucan and vitamin E were supplemented in the feed of lactating sows, the addition of β -glucan increased the feed intake of the lactating sows, and the feed intake was the highest when 0.1% β -glucan was added.

Litter performance

The effects of β -glucan and vitamin E supplementation in the lactating sow diet on litter performance during lactation are shown in Table 3. There were no significant differences in the number of piglets after cross fostering, the number of weaning pigs, litter birth weight, or piglet birth weight. However, there was an increasing trend in piglet weight at weaning (BG, p=0.07), litter weight at the 21st day of lactation (BG, p=0.07) and litter weight gain (BG, p=0.08) in treatments supplemented with 0.1% β -glucan compared to treatments supplemented with 0.2% β -glucan. In addition, piglet weight at weaning was significantly higher in all treatments added with β -glucan or vitamin E than in the control (Diet, p<0.01).

Lactating sows must be supplied with sufficient nutrients to meet the nutritional requirements for body maintenance immediately after farrowing, and an ad libitum feeding system was implemented after the feed intake was maximized (Eissen et al.,

2000). Previous studies have reported that ad libitum feeding during lactation increased the total feed intake of piglets (Sørensen, 2005). In addition, litter weight gain increased in cases of lactating sows with a high feed intake. Lactating sows need sufficient nutrients for milk production. When the feed intake of lactating sows was increased, their milk production eventually improved.

Szuba-Trznadel et al. (2014) reported that there was no significant difference in the growth performance of piglets even when 0.02% or 0.03% β -glucan was added to lactating and gestating sow diets. Likewise, the addition of varying levels β -glucan to the sow diet (0.5%, 1%, 2%, 0.15%, and 0.3%) had no effect on piglet weight or on the number of weaning pigs (Mahan, 2000). However, the ADG and weaning weight of piglets were improved when 4% β -glucan extracted from yeast was supplemented in lactating and gestating sow diets (Fuchs et al., 2007). In addition, Li et al. (2006) reported that the ADG of piglets was increased when 0.005% β -glucan was added to a lactating sow diet. According to experiments by Hiss and Sauerwein (2003), the ADG and weaning weight of piglets were improved when 0.015%-0.03% β -glucan was added to the lactating sow diet. It is commonly known that the addition of more than 44 IU of vitamin E to diets does not adversely affect the health status and reproductive performance of sows (Mahan, 1991; Mahan, 1994). Shelton et al. (2014) reported that there was no significant difference in the reproductive performance of sows and the growth performance of piglets, even when vitamin E (15, 30, 45, 60 IU D- α -TAc and 44, 66 IU DL- α -TAc) was added to the gestating and lactating sow diets. Mahan et al. (2000) reported that the addition of 30 or 60 IU of vitamin E to the sow diet did not show a positive effect on the growth performance of the piglets.

In the present experiment, piglet weight at weaning (BG, $p=0.07$), litter weight on the 21st day of lactation (BG, $p=0.07$) and litter weight gain (BG, $p=0.08$) exhibited an increasing trend when β -glucan was added to the lactating sow diet. This was because pig milk production increased due to the high feed intake of lactating sows, as observed in previous studies. Accordingly, it was hypothesized that the growth performance of the piglets increased when they received sufficient nutrients. Additionally, the effect of adding vitamin E was not observed in the current

experiment, which is similar to previous studies by Mahan (2000) and Shelton et al. (2014).

In conclusion, the addition of 0.1% β -glucan in the diets of lactating sows had a positive effect on piglet weight at weaning, litter weight at the 21st day of lactation and litter weight gain.

Blood profiles

The effects of β -glucan and vitamin E supplementation in the lactating sow diet on blood profiles during lactation are shown in Table 4. There was no significant difference in the concentration of selenium in lactating sows and piglets. However, treatments supplemented with 110 IU vitamin E/kg compared to treatments supplemented with non-additional vitamin E at 21 days of lactation showed a significantly higher vitamin E concentration (VE, $p < 0.01$) in lactating sows and an increasing trend in vitamin E concentration (VE, $p = 0.09$) in piglets. In addition, vitamin E concentration at 21 days of lactation was significantly higher in all treatments added with β -glucan or vitamin E than in the control (Diet, $p < 0.01$).

When vitamin E was supplemented at different concentrations (44 IU and 66 IU) starting on 70 days of gestation and ending prior to weaning in a study by Shelton et al. (2014), the concentration of α -tocopherol in the plasma of sows increased linearly at 100 days of gestation, within 24 hours of parturition and during weaning. In addition, Mahan (1994) also showed that the concentration of α -tocopherol in the serum of lactating sows and piglets was significantly increased as vitamin E was added at different levels (22 IU, 44 IU, and 66 IU) in the lactating sow diet. In a study by Umesiobi (2009), the concentration of α -tocopherol significantly increased as the level of vitamin E addition increased (0 IU, 40 IU, and 70 IU) in the feed of 1st parity sows and 2nd parity sows. Wang et al. (2017) conducted an experiment comparing a control group in which 44 IU/kg vitamin E was added to a basal diet and a treatment group in which a total of 250 IU/kg of vitamin E was added from the 107th day of gestation until the piglets were weaned. Their results showed that the concentration of α -tocopherol in the blood of lactating sows and piglets was significantly higher in

the treatment group than in the control ($p < 0.01$).

Due to limited movement of nutrients from the mother to the fetus through the placenta during gestation, piglets are deficient in vitamin E immediately after birth, so α -tocopherol must be supplied through the colostrum and pig milk of sows (1991). Insufficient intake of milk by piglets may lead to a decrease in serum vitamin E levels during weaning, which may increase oxidative status and susceptibility to disease.

In the current experiment, we found that the concentration of α -tocopherol in the blood of lactating sows and piglets was increased when vitamin E was added to the lactating sow diet. The increase in the concentration of vitamin E in the blood was consistent with the results of previous studies by Mahan et al. (1994), Shelton et al. (2014), Umesiobi et al. (2009) and Wang et al. (2017). This showed that when vitamin E was additionally supplied to lactating sows, the effect was that the concentration of vitamin E in the blood increased through the sows. The concentration of vitamin E in the piglets was also increased, as in previous studies by Mahan et al. (2000) and Wang et al. (2017). The results showed that the increase in the concentration of vitamin E in the blood of lactating sows is correlated to the level of vitamin E added to the lactating sow diet. This suggests that vitamin E was also well delivered to piglets through pig milk. Additionally, it can be expected that this will have a positive effect on the reduction of oxidative stress and disease susceptibility of piglets. In addition, it can be reasoned that this helps to improve immunity not only in lactating sows but also in piglets (Wang et al., 2017).

In conclusion, the addition of 110 IU vitamin E/kg to lactating sow feed had a positive effect on the concentration of vitamin E in the blood of lactating sows and piglets.

Immune response

The effects of β -glucan and vitamin E supplementation in the lactating sow diet on immune response during lactation are shown in Table 5. The results show that there was no significant difference in the concentration of lymphocytes and IL-6 in

lactating sows and piglets. However, adding 0.1% β -glucan compared to 0.2% β -glucan showed a lower trend in TNF- α concentration in lactating sows (BG, $p=0.06$) and in piglets (BG, $p=0.09$) on the 21st day of lactation.

In general, piglet immunity is derived from the absorption of immune proteins in the colostrum and from immune proteins synthesized in the body after 35 days. Lymphocytes are part of the adaptive immune system and account for 20-40% of white blood cells. TNF- α and IL-6 secretion indicates excessive inflammatory responses, including the expression of inflammatory cytokines. TNF- α is a proinflammatory cytokine that activates adaptive immunity against viral infections. TNF- α blood levels have been used as an indicator of inflammation in humans and animals. Generally, proinflammatory cytokines such as TNF- α negatively affect animal growth and well-being when they are increased. However, many cytokines play a very important role in enhancing the innate immune response and directing adaptive immunity against responses dominated by Th1 or Th2 cells. β -glucan is well known as an important stimulator of the cellular branch of the immune response and improves biological and immunological status (Vetvicka et al., 2014). Additionally, it partially inhibits or reduces the increase in TNF- α production upon endotoxin and lipopolysaccharide (LPS) challenge (Li et al., 2006; Lee et al., 2021).

TNF- α blood levels ($p<0.05$) were decreased on the 7th day when weaning pigs were fed a 0.05% yeast cell wall product containing β -glucan (Lee et al., 2021). This was explained by the increased antibody production observed after immune system stimulation as a result of binding with the β -glucan receptor and by triggering a cytokine cascade and improving macrophage function. The ileal expression of TNF- α mRNA was higher in weaning pigs fed 10 g/d of seaweed extract, mainly containing laminarians composed of β -glucan, than in control weaning pigs ($p<0.01$) (Li et al., 2005). Li et al. (2006) conducted a 2x2 factorial experiment in weaning pigs with and without LPS inoculation and with or without β -glucan addition (0% or 0.005%). The results show that TNF- α levels ($p<0.05$) were lower in weaning pigs supplemented with 0.005% β -glucan at 3, 9, 18, and 24 hours, regardless of LPS inoculation. In addition, TNF- α levels were increased by LPS at all time points except at 0 hour

($p < 0.05$). When weaning pigs inoculated with LPS were fed a diet supplemented with β -glucan extracted from 0.005% baker's yeast (*Saccharomyces cerevisiae*), TNF- α levels in the blood were lower at 3 hours ($p < 0.05$) and 4.5 hours after injection than they were in control pigs (Li et al., 2006). As a result of an experiment on the effect of adding vitamin C and β -glucan to weaning pigs after endotoxin challenge, the spleen of weaning pigs fed 2.5% β -glucan expressed a higher relative abundance of TNF- α mRNA compared to the control pigs ($p < 0.01$) (Eicher et al., 2006).

In pigs, an increase in inflammatory cytokines such as TNF- α resulted in the distribution of nutrients to the immune system rather than growth, leading to decreased net muscle accumulation and growth (Li et al., 2005). This indicated that since the TNF- α concentration in the treatments supplemented with 0.1% β -glucan showed a decreasing trend, more nutrients were distributed for animal growth. This is evident from the significantly higher ADFI of lactating sows in the treatments supplemented with 0.1% β -glucan compared to 0.2% β -glucan (BG, $p < 0.01$) and from the increase in piglet weight at weaning (BG, $p = 0.07$), litter weight at the 21st day of lactation (BG, $p = 0.07$) and litter weight gain (BG, $p = 0.08$).

In conclusion, the TNF- α concentration was thought to be decreased as the immune status of the sows and piglets was improved when 0.1% β -glucan was added to the lactating sow diet.

Milk composition

The effects of β -glucan and vitamin E supplementation in the lactating sow diet on milk composition during lactation are shown in Table 6. The results show that there were no significant differences in casein, protein, fat, total solids, SNF, or lactose in the milk of lactating sows.

Various factors, such as breed, sow health, feeding program, nutritional level, and environmental conditions, affect sow milk composition and production. Milk composition has been shown to affect the growth of piglets (Shen et al., 2011). Nutrient requirements in lactating sows are significantly higher than those during the gestation period. Lactating sows were generally fed ad libitum for optimal milk

production, maintenance, and maternal recovery.

In the present experiment, there was no significant difference in the milk composition of sows between the treatments after the addition of β -glucan and vitamin E. Since the nutrients accumulating in the body of the sows were preferentially transferred to the pig's milk, the effect on the feed intake during the lactation period was small. Thus, we concluded that the components of the sow milk were not affected.

As shown here, Shen et al. (2011) reported that feeding *Saccharomyces cerevisiae* fermentation product to sows during gestation and lactation did not affect the composition of the colostrum and milk. In the study by Chen et al. (2020), colostrum and milk components were not affected when *Saccharomyces cerevisiae* fermentation product was added in a subtropical climate.

In conclusion, the addition of β -glucan with vitamin E to the feed of lactating sows did not have a positive effect on the components of sow milk.

CONCLUSION

This study was conducted to evaluate the effects of β -glucan with vitamin E supplementation on the physiological response, litter performance, blood profiles, immune response and milk composition of lactating sows.

The addition of 0.1% β -glucan improved the growth performance of the piglets by providing them with sufficient nutrients. This was because the production of pig milk improved as the feed intake of the sows increased. In addition, treatments with 0.1% β -glucan induced a lower TNF- α concentration than the treatments with 0.2% β -glucan. This meant that the inflammatory response in the lactating sows and piglets was reduced and the health status was improved, which was thought to be the result of the improvement in the growth performance of the piglets. Furthermore, the addition of 110 IU vitamin E/kg increased the concentration of α -tocopherol in the blood of the sows and piglets, indicating that addition of vitamin E was better delivered to the sows and piglets. However, as the feed intake of sows increased, a more positive effect on the growth performance of the piglets was found when β -glucan was added at 0.1% rather than 0.2%. Therefore, adding 0.1% β -glucan and 110 IU vitamin E/kg to the feed of the sows was beneficial to the growth performance of the piglets by increasing the feed intake of the sows and efficiently supplying vitamin E to the sows and piglets.

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Table 1. Formula and chemical compositions of the experimental diets during the lactation phase

Items	Treatment ¹⁾				
	CON	LB	LBE	HB	HBE
Ingredient (%)					
Corn	66.19	66.12	66.10	66.09	66.04
Soybean meal-46%	15.32	15.29	15.28	15.22	15.23
Wheat	5.00	5.00	5.00	5.00	5.00
Wheat bran	5.00	5.00	5.00	5.00	5.00
Vitamin E ²⁾	0.00	0.00	0.02	0.00	0.02
β -glucan ²⁾	0.00	0.10	0.10	0.20	0.20
Tallow	3.58	3.58	3.59	3.58	3.60
L-lysine HCl (78%)	0.43	0.43	0.43	0.43	0.43
DL-methionine (99%)	0.07	0.07	0.07	0.07	0.07
L-threonine (99%)	0.11	0.11	0.11	0.11	0.11
Tryptophan (10%)	0.31	0.31	0.31	0.31	0.31
MDCP	1.86	1.86	1.86	1.86	1.86
Limestone	1.33	1.33	1.33	1.33	1.33
Vitamin. Mix ³⁾	0.10	0.10	0.10	0.10	0.10
Mineral. Mix ⁴⁾	0.10	0.10	0.10	0.10	0.10
Choline chloride-50%	0.10	0.10	0.10	0.10	0.10
Salt	0.50	0.50	0.50	0.50	0.50
Sum	100.00	100.00	100.00	100.00	100.00
Chemical composition⁵⁾					
ME (kcal/kg)	3300.00	3300.00	3300.00	3300.00	3300.00
Crude protein (%)	13.43	13.43	13.43	13.43	13.43
Lysine (%)	0.96	0.96	0.96	0.96	0.96
Methionine (%)	0.29	0.29	0.29	0.29	0.29
Cysteine (%)	0.27	0.27	0.27	0.27	0.27
Threonine (%)	0.61	0.61	0.61	0.61	0.61
Tryptophan (%)	0.17	0.17	0.17	0.17	0.17
Calcium (%)	0.90	0.90	0.90	0.90	0.90
Total phosphorus (%)	0.70	0.70	0.70	0.70	0.70

MDCP, mono-dicalcium phosphate; ME, metabolizable energy; SBM, soybean meal

¹⁾ CON: corn-SBM based diet, LB: corn-SBM based diet+0.1% β -glucan, LBE: corn-SBM based diet+0.1% β -glucan+110 IU vitamin E/kg, HB: corn-SBM based diet+0.2% β -glucan, HBE: corn-SBM based diet+0.2% β -glucan+110 IU vitamin E/kg

²⁾ β -glucan and vitamin E products were provided by E&T company (E&T Co., Ltd, Daejeon, South Korea).

³⁾ Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 12,000 IU; Vitamin D₃, 1,200 IU; Vitamin E, 68 IU; Vitamin K₃, 5 mg; Thiamin, 2.6 mg; Ribo flavin, 8 mg; Calcium pantothenic acid, 26 mg; Niacin, 60 mg; D-Biotin, 0.5 mg; Vitamin B₆, 6 mg; Vitamin B₁₂, 0.025 mg.

⁴⁾ Provided the following quantities of minerals per kg of complete diet: Fe, 120 mg; Cu, 6 mg; Mn, 60 mg; I, 0.75 mg; Se, 0.3 mg; Zn, 46 mg.

⁵⁾ Calculated value.

Table 2. Effects of β -glucan with vitamin E supplementation on the physiological response in lactating sows

Items	Treatment ¹⁾					SEM ²⁾	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Body weight, kg										
24 hours postpartum	231.90	231.13	236.39	235.76	231.14	4.302	0.87	0.97	0.97	0.62
21 st day of lactation	232.88	237.81	234.54	232.68	228.21	4.857	0.97	0.61	0.73	0.95
Changes (0-21d)	0.98	6.68	-1.85	-3.08	-2.93	2.612	0.84	0.36	0.49	0.47
Backfat thickness, mm										
24 hours postpartum	22.00	22.95	23.00	23.45	22.45	0.693	0.59	0.98	0.77	0.75
21 st day of lactation	20.65	20.60	21.65	21.85	20.95	0.697	0.73	0.86	0.96	0.55
Changes (0-21d)	-1.35	-2.35	-1.35	-1.60	-1.50	0.358	0.70	0.71	0.51	0.59
ADFI, kg/d	5.25	6.59	6.14	6.29	6.01	0.112	<0.01	<0.01	0.17	0.25
WEI, day	3.41	3.91	3.72	3.66	3.85	0.126	0.30	0.84	0.99	0.51

BG, β -glucan; VE, vitamin E; ADFI, average daily feed intake; WEI, weaning-to-estrus interval; SBM, soybean meal

¹⁾ CON: corn-SBM based diet, LB: corn-SBM based diet+0.1% β -glucan, LBE: corn-SBM based diet+0.1% β -glucan+110 IU vitamin E/kg, HB: corn-SBM based diet+0.2% β -glucan, HBE: corn-SBM based diet+0.2% β -glucan+110 IU vitamin E/kg

²⁾ Standard error of the mean

Table 3. Effects of β -glucan with vitamin E supplementation on litter performance

Items	Treatment ¹⁾					SEM ²⁾	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
No. of sows	10.00	10.00	10.00	10.00	10.00	-	-	-	-	-
No. of piglets										
Initial	11.30	11.60	11.60	12.00	11.90	0.310	0.97	0.34	0.13	0.13
21 st day of lactation	10.00	10.40	10.50	10.60	10.30	0.251	0.75	0.38	0.29	0.22
Litter weight, kg										
Initial	14.90	17.01	15.91	16.55	15.94	0.443	0.20	0.82	0.40	0.81
21 st day of lactation	56.34	66.42	64.53	65.94	61.90	1.765	0.11	0.07	0.13	0.28
Litter weight gain	41.43	49.41	48.62	49.39	45.96	1.638	0.27	0.08	0.11	0.22
Piglet weight, kg										
Initial	1.32	1.47	1.37	1.38	1.43	0.031	0.25	0.89	0.78	0.37
21 st day of lactation	5.70	6.33	6.45	6.24	6.01	0.099	<0.01	0.07	0.18	0.10
Piglet weight gain	4.37	4.87	4.79	4.85	4.57	0.862	0.62	0.64	0.77	0.89

BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON: corn-SBM based diet, LB: corn-SBM based diet+0.1% β -glucan, LBE: corn-SBM based diet+0.1% β -glucan+110 IU vitamin E/kg, HB: corn-SBM based diet+0.2% β -glucan, HBE: corn-SBM based diet+0.2% β -glucan+110 IU vitamin E/kg

²⁾ Standard error of the mean

Table 4. Effects of β -glucan with vitamin E supplementation on blood profiles in lactating sows and piglets

Items	Treatment ¹⁾					SEM ²⁾	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Sow										
Selenium, $\mu\text{g/L}$										
Initial	----- 154.2 -----					-	-	-	-	-
21 st day of lactation	208.60	202.00	195.50	208.50	216.00	4.036	0.76	0.17	0.96	0.47
Vitamin E (Tocopherol), $\mu\text{mol/L}$										
Initial	-----3.5-----					-	-	-	-	-
21 st day of lactation	7.05	7.50	10.78	7.88	9.50	0.399	0.06	0.60	<0.01	0.32
Piglet										
Selenium, $\mu\text{g/L}$										
Initial	----- 67.2 -----					-	-	-	-	-
21 st day of lactation	90.20	108.40	85.67	88.75	90.80	4.461	0.77	0.50	0.34	0.26
Vitamin E (Tocopherol), $\mu\text{mol/L}$										
Initial	-----9.4-----					-	-	-	-	-
21 st day of lactation	8.60	9.70	19.45	15.15	15.93	1.287	0.53	0.64	0.09	0.10

BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON: corn-SBM based diet, LB: corn-SBM based diet+0.1% β -glucan, LBE: corn-SBM based diet+0.1% β -glucan+110 IU vitamin E/kg, HB: corn-SBM based diet+0.2% β -glucan, HBE: corn-SBM based diet+0.2% β -glucan+110 IU vitamin E/kg

²⁾ Standard error of the mean

Table 5. Effects of β -glucan with vitamin E supplementation on the immune response in lactating sows and piglets

Items	Treatment ¹⁾					SEM ²⁾	p-value				
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE	
Sow											
Lymphocyte, %											
Initial	-----30.2-----					-	-	-	-	-	-
21 st day of lactation	42.50	38.43	42.38	40.67	42.30	1.233	0.64	0.73	0.38	0.71	
TNF-α, ng/mL											
Initial	-----1.06-----					-	-	-	-	-	
21 st day of lactation	1.45	2.12	2.20	2.33	2.24	0.124	0.89	0.06	0.89	0.17	
IL-6, ng/mL											
Initial	-----2.42-----					-	-	-	-	-	
21 st day of lactation	3.61	3.58	3.23	4.41	1.87	0.483	0.78	0.82	0.23	0.36	
Piglet											
Lymphocyte, %											
Initial	-----38.1-----					-	-	-	-	-	
21 st day of lactation	71.70	67.78	51.11	74.87	78.37	4.709	0.76	0.13	0.55	0.36	
TNF-α, ng/mL											
Initial	-----1.35-----					-	-	-	-	-	
21 st day of lactation	0.45	0.32	1.39	1.67	1.60	0.230	0.12	0.09	0.26	0.21	
IL-6, ng/mL											
Initial	-----1.74-----					-	-	-	-	-	
21 st day of lactation	0.83	1.07	0.78	1.60	1.35	0.215	0.70	0.67	0.12	0.74	

BG, β -glucan; VE, vitamin E; SBM, soybean meal

¹⁾ CON: corn-SBM based diet, LB: corn-SBM based diet+0.1% β -glucan, LBE: corn-SBM based diet+0.1% β -glucan+110 IU vitamin E/kg, HB: corn-SBM based diet+0.2% β -glucan, HBE: corn-SBM based diet+0.2% β -glucan+110 IU vitamin E/kg

²⁾ Standard error of the mean

Table 6. Effects of β -glucan with vitamin E supplementation on milk composition in lactating sows

Items	Treatment ¹⁾					SEM ²⁾	p-value			
	CON	LB	LBE	HB	HBE		Diet	BG	VE	BG*VE
Casein, %										
24 hours postpartum	-----5.15-----					-	-	-	-	-
21 st day of lactation	3.65	3.62	3.63	3.70	3.70	0.044	0.91	0.50	0.99	0.94
Protein, %										
24 hours postpartum	-----7.28-----					-	-	-	-	-
21 st day of lactation	4.46	4.39	4.43	4.50	4.59	0.062	0.91	0.38	0.67	0.88
Fat, %										
24 hours postpartum	-----7.80-----					-	-	-	-	-
21 st day of lactation	5.73	6.60	7.11	6.69	6.61	0.232	0.10	0.69	0.68	0.58
Total solid, %										
24 hours postpartum	-----21.47-----					-	-	-	-	-
21 st day of lactation	17.46	18.31	18.69	18.41	18.35	0.247	0.14	0.83	0.79	0.70
SNF, %										
24 hours postpartum	-----12.50-----					-	-	-	-	-
21 st day of lactation	11.19	11.01	10.84	11.06	11.08	0.057	0.19	0.27	0.57	0.47
Lactose, %										
24 hours postpartum	-----4.38-----					-	-	-	-	-
21 st day of lactation	5.89	5.79	5.66	5.78	5.69	0.041	0.14	0.89	0.25	0.85

BG, β -glucan; VE, vitamin E; SNF, solid not fat; SBM, soybean meal.

¹⁾ CON: corn-SBM based diet, LB: corn-SBM based diet+0.1% β -glucan, LBE: corn-SBM based diet+0.1% β -glucan+110 IU vitamin E/kg, HB: corn-SBM based diet+0.2% β -glucan, HBE: corn-SBM based diet+0.2% β -glucan+110 IU vitamin E/kg

²⁾ Standard error of the mean

Overall Conclusion

This study was carried out to evaluate the effects of β -glucan with vitamin E in swine diets. Therefore, three experiments were conducted to investigate 1) the effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, fecal microbiota, fecal score, and nutrient digestibility in weaning pigs, 2) the effects of β -glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, pork quality, pork flavor, and economic benefit in growing and finishing pigs, and 3) the effects of β -glucan with vitamin E supplementation on the physiological response, litter performance, blood profiles, immune response, and milk composition in lactating sows.

In experiment 1, a significant decrease in YM and Proteobacteria and a tendency for Lactobacillus to increase compared to the control was shown when 0.1% β -glucan and 0.02% vitamin E were added. The fecal score in weaning pigs was lower in the treatments supplemented with 0.1% or 0.2% β -glucan and 0.02% vitamin E compared to the control. In addition, vitamin E was better supplied to weaning pigs by increasing the concentration of α -tocopherol in the blood of weaning pigs when 0.02% vitamin E was supplemented. Therefore, the addition of 0.1% β -glucan with 0.02% vitamin E to weaning pig feed is thought to have a positive effect on the growth performance of weaning pigs by improving the intestinal microbial composition and reducing the occurrence of diarrhea while efficiently supplying vitamin E.

In experiment 2, the ADG and feed efficiency were improved compared to the control when β -glucan or vitamin E was added. Supplementing 0.05% β -glucan significantly increased lymphocyte concentration compared to the addition of 0.1% β -glucan and the content of vitamin E in the blood increased when 0.02% vitamin E was added. The HBE treatment added with 0.1% β -glucan and 0.02% vitamin E showed the most economic effect because it had the shortest days to market weight and the lowest total feed cost. However, carcass traits and meat quality were not affected by β -glucan or vitamin E. Therefore, the addition of 0.1% β -glucan with 0.02% vitamin E in growing and finishing pig's diet showed great growth performance and

economic effect by supplying vitamin E efficiently and by improving the health condition of pigs due to β -glucan

In experiment 3, the addition of 0.1% β -glucan improved the growth performance of the piglets by providing them with sufficient nutrients. This was because the production of pig milk improved as the feed intake of the sows increased. In addition, treatments with 0.1% β -glucan showed a lower TNF- α concentration than the treatments with 0.2% β -glucan in lactating sows and piglets. This meant that the inflammatory response in the lactating sows and piglets was reduced and the health status was improved, which was thought to be the result of the improvement in the growth performance of the piglets. Furthermore, the addition of 110 IU vitamin E/kg increased the concentration of α -tocopherol in the blood of the sows and piglets, indicating that addition of vitamin E was better delivered to the sows and piglets. However, as the feed intake of sows increased, a more positive effect on the growth performance of the piglets was found when β -glucan was added at 0.1% rather than 0.2%. Therefore, adding 0.1% β -glucan with 110 IU vitamin E/kg to the feed of the sows was beneficial to the growth performance of the piglets by increasing the feed intake of the sows and efficiently supplying vitamin E to the sows and piglets.

Consequently, these results implied that the most effective results were obtained when 0.1% β -glucan with 0.02% vitamin E were added in weaning pig, growing and finishing pig, and lactating sow diets.

Summary in Korean

본 실험은 양돈사료 내 베타글루칸과 비타민 E의 첨가효과를 알아보기 위하여 수행되었다. 총 3개의 실험으로 구성되어 있으며, 1) 이유자돈 사료 내 베타글루칸과 비타민 E의 첨가가 이유자돈의 성장성적, 혈액성상, 면역성상, 분내 미생물, 설사지수 및 영양소 소화율에 미치는 영향, 2) 육성비육돈 사료 내 베타글루칸과 비타민 E의 첨가가 육성비육돈의 성장성적, 혈액성상, 면역성상, 돈육품질, 돈육풍미 및 경제성 분석에 미치는 영향, 3) 포유돈 사료 내 베타글루칸과 비타민 E의 첨가가 포유돈의 생리적 반응, 포유자돈의 성장성적, 혈액성상, 면역성상 및 돈유성분에 미치는 영향을 평가하였다.

실험 1. 이유자돈 사료 내 베타글루칸과 비타민 E의 첨가가 이유자돈의 성장성적, 혈액성상, 면역성상, 분내 미생물, 설사지수 및 영양소 소화율에 미치는 영향

본 연구는 이유자돈 사료 내 베타글루칸과 비타민 E의 첨가가 이유자돈의 성장성적, 혈액성상, 면역성상, 분내 미생물, 설사지수 및 영양소 소화율에 미치는 영향을 규명하기 위해 수행되었다. 본 실험은 삼원교잡종 ([Yorkshire × Landrace] × Duroc) 이유자돈 200두를 공시하여 5처리, 4반복, 반복 당 10두로 체중과 성별을 고려하여 난괴법 (Randomized Complete Block Design; RCBD)으로 배치하였다. 실험은 총 6주 동안 (자돈 전기 3주와 자돈 후기 3주) 수행하였다. 처리구는 베타글루칸과 비타민 E의 첨가수준에 따라 자돈전기와 후기 동일하게 1) CON : 옥수수-대두박 위주의 기초 사료, 2) LB : 기초사료 + 베타글루칸 0.1%, 3) LBE : 기초사료 + 베타글루칸 0.1% + 비타민 E 0.02%, 4) HB : 기초사료 + 베타글루칸 0.2%,

5) HBE : 기초사료 + 베타글루칸 0.2% + 비타민 E 0.02%로 나뉘었다. 성장 성적의 경우, 이유자돈 사료 내 베타글루칸과 비타민 E를 첨가하였을 때 이유자돈의 체중, 일당증체량, 사료섭취량이 증가하였다. 분변 내 미생물의 경우, 대조구 대비 0.1% 베타글루칸과 0.02% 비타민 E를 첨가한 처리구에서 효모&곰팡이와 Proteobacteria의 유의적인 감소와 Lactobacillus가 증가하는 경향을 보였다. 설사지수의 경우, 0.1% 및 0.2% 베타글루칸과 0.02% 비타민 E를 첨가한 처리구가 대조구 대비 3주차 및 6주치의 설사 지수가 유의적으로 더 낮았다. 비타민 E 0.02% 첨가는 이유자돈의 혈액 내 α -tocopherol의 농도를 증가시킴으로써 비타민 E가 이유자돈 체내에 더 잘 공급되게 하였다. 하지만, 혈액 내 셀레늄, TNF- α , IL-6, 림프구 농도 및 이유자돈의 영양소 소화율에서는 처리구 간 유의적인 차이가 나타나지 않았다.

결론적으로, 이유자돈 사료 내 베타글루칸 0.1%와 비타민 E 0.02%를 첨가하였을 때 장내 미생물 성상을 개선하여 설사 발생이 감소함에 따라 이유자돈의 성장성적에 긍정적인 효과를 미치는 것으로 사료된다.

실험 2. 육성비육돈 사료 내 베타글루칸과 비타민 E의 첨가가 육성비육돈의 성장성적, 혈액성상, 면역성상, 돈육품질, 돈육풍미 및 경제성 분석에 미치는 영향

본 연구는 육성비육돈 사료 내 베타글루칸과 비타민 E의 첨가가 육성비육돈의 성장성적, 혈액성상, 면역성상, 돈육품질, 돈육풍미 및 경제성 분석에 미치는 영향을 규명하기 위해 수행되었다. 본 실험은 삼원교잡종 [(Yorkshire × Landrace) × Duroc] 육성돈 140두를 공시하여 5처리, 4반복, 반복 당 7두로 성별과 체중에 따라 난괴법 (Randomized Complete Block Design; RCBD)으로 배치하였다. 육성전기 (3주), 육성후기 (3주), 비육전기 (3주), 비육후기 (3주)로 나누어 총 12주 동안 사양실험을 수행하였다. 육성 전기/후기 및 비육 전기/후기 실험사료 및 처리구는 베타글루칸과 비타민 E의 첨가에 따라 1) CON : 옥수수-대두박 위주의 기초 사료, 2) LB : 기초사료 + 베타글루칸 0.05%, 3) LBE : 기초사료 + 베타글루칸 0.05% + 비타민 E 0.02%, 4) HB : 기초사료 + 베타글루칸 0.1%, 5) HBE : 기초사료 + 베타글루칸 0.1% + 비타민 E 0.02%로 나뉘었다. 실험 결과, 일당증체량 및 사료효율은 대조구 대비 육성비육돈 사료 내 베타글루칸 또는 비타민 E를 첨가하였을 때 향상되었다. 베타글루칸 0.05% 첨가는 베타글루칸 0.1% 넣었을 때와 비교하여 유의적으로 lymphocyte의 농도를 증가시켰고 비타민 E 0.02% 첨가하였을 때 혈중 내 비타민 E의 함량이 증가하였다. 경제성 분석의 경우, 베타글루칸 0.1%와 비타민 E 0.02%를 첨가한 HBE 처리구가 수치상으로 출하도달 일령이 가장 짧고 총 사료비가 가장 낮았기 때문에 가장 경제적인 효과를 보였다. 베타글루칸 및 비타민 E의 첨가가 IMP의 함량을 증가시킨 것으로 볼 때 돈육의 풍미를 향상시킨 것에 긍정적인 역할을 하였다. 하지만, 도체특성 및 육질의 품질에서는 유의적인 차이가 나타나지 않았다.

결론적으로, 육성비육돈 사료 내 베타글루칸 0.1%와 비타민 E 0.02%를 첨가하였을 때 비타민 E가 육성비육돈에게 추가적으로 잘 전달되었고 베타글루칸으로 인해 육성비육돈의 건강상태가 좋아져 성장성적에 긍정적인 효과를 미치고 경제적인 효과를 보인 것이라고 사료된다.

실험 3. 포유모돈 사료 내 베타글루칸과 비타민 E 의 첨가가 포유모돈의 생리적 반응, 포유자돈 성장성적, 혈액성상, 면역성상 및 돈유성분에 미치는 영향

본 연구는 포유모돈 사료 내 베타글루칸과 비타민 E의 첨가가 포유모돈의 생리적 반응, 포유자돈 성장성적, 혈액성상, 면역성상 및 돈유 성분에 미치는 영향을 규명하기 위해 수행되었다. 본 실험은 2원 교잡종 (Yorkshire × Landrace) F1 모돈(평균 체중 $233.6 \pm 4.3\text{kg}$) 50두를 공시하여, 5처리, 10반복, 반복 당 1두씩을 완전임의 배치법 (CRD: completely randomized design)으로 배치하여 실시하였다. 처리구는 베타글루칸과 비타민 E의 첨가수준에 따라 1) CON: 옥수수-대두박 위주의 기초 사료, 2) LB: 기초사료 + 베타글루칸 0.1%, 3) LBE: 기초사료 + 베타글루칸 0.1% + 비타민 E 110 IU/kg, 4) HB: 기초사료 + 베타글루칸 0.2%, 5) HBE: 기초사료 + 베타글루칸 0.2% + 비타민 E 110 IU/kg로 나뉘었다. 실험 결과, 분만 24시간 이내, 포유 21일차 포유모돈의 체중 및 등지방 두께에서는 통계적인 유의적 차이가 나타나지 않았다 ($p>0.05$). 또한, 포유모돈의 재귀발정일에서도 통계적인 유의차가 나타나지 않았다 ($p>0.05$). 하지만, 포유모돈의 사료섭취량에서는 대조구 대비 베타글루칸이 첨가된 모든 처리구에서 유의적으로 높았으며 (Diet, $p<0.01$) 베타글루칸 0.1% 첨가한 처리구가 베타글루칸 0.2% 첨가한 처리구보다 유의적으로 더 높았다 (BG, $p<0.01$). 베타글루칸 0.1% 첨가한 처리구가 베타글루칸 0.2% 첨가한 처리구보다 베타글루칸에 의한 포유 21일령 복당 자돈체중 (BG, $p=0.07$), 복당 자돈 일당 증체량 (BG, $p=0.08$) 및 자돈 이유체중 (BG, $P=0.07$)에서 더 높은 경향이 나타났다. 또한, 포유 21일령에 비타민 E 110 IU/kg 첨가한 처리구가 비타민 E를 첨가하지 않은 처리구에 비해 포유모돈의 혈액 내 비타민 E 농도 (VE, $p<0.01$)가 유의적으로 더 높게 나타났으며 포유자돈의 혈액 내 비타

민 E 농도 (VE, $p=0.09$)에서도 더 높은 경향이 나타났다. 또한, 포유 21일령에는 베타글루칸 0.1% 첨가한 처리구가 베타글루칸 0.2% 첨가한 처리구에 비해 베타글루칸에 의한 포유모돈의 혈액 내 TNF- α 농도 (BG, $p=0.06$)와 포유 자돈의 혈액 내 TNF- α 농도 (BG, $p=0.09$)에서 더 낮은 경향이 나타났다.

결론적으로, 베타글루칸 0.1% 첨가는 포유모돈의 사료섭취량을 증가시켜 돈유 생산량이 증가함에 따라 포유자돈에게 충분한 영양소를 전달함으로써 포유자돈의 성장성적을 향상시켰다. 또한, 베타글루칸 0.1% 첨가한 처리구는 0.2% 첨가 처리구에 비해 TNF- α 농도가 더 낮게 나타났다. 이는 포유모돈과 포유자돈에 있어 염증반응이 줄어들어 건강상태가 좋아진 것을 의미하므로 포유자돈 성장성적이 향상된 결과로 나타난 것으로 사료된다. 게다가, 비타민 E 110 IU/kg 첨가는 포유모돈 및 포유자돈의 혈액 내 α -tocopherol의 농도를 증가시킴으로써 비타민 E가 포유모돈 및 포유 중인 자돈들에게 추가적으로 더 잘 공급되었음을 의미한다. 그러나 베타글루칸 0.2%보다 0.1%를 첨가하였을 때 모돈의 사료섭취량이 증가함에 따라 포유자돈의 성장성적에도 더 긍정적인 영향을 미치는 것으로 나타났다. 따라서 본 실험 결과, 포유모돈의 사료에 베타글루칸 0.1%와 비타민 E 110 IU/kg를 첨가하면 포유모돈의 사료섭취량 증가와 포유모돈 및 포유자돈에게 비타민 E의 효율적인 공급에 따라 포유자돈의 성장성적에 유익한 것으로 나타났다.

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