A Thesis
For the Degree of Master of Science

Effect of zinc oxide and probiotics on growth performance, immune response, diarrhea index and fecal microflora in weaning pigs

이유자돈 사료 내 산화아연과 생균제 첨가가 자돈의 성장성적, 면역성상, 설사빈도 및 분변 내 미생물에 미치는 영향

August, 2021

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이 논문을 농학석사 학위논문으로 제출함

2021 년 4 월

서울대학교 대학원 농생명공학부

문 건 웅

문건웅의 농학석사 학위논문을 인준함

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Summary

It is commonly to add zinc oxide to piglet feed to prevent diarrhea caused by weaning stress. Most of countries are allowing excessive amount of zinc oxide supplementation and is added pharmacologically to 2,000 ~ 4,000 ppm. This causes a severe problem in environmental pollution subsequently many countries try to find alternative feed supplements such as probiotics, prebiotics, acidifier, minerals instead of zinc oxide. Probiotics are the most suggested material among feed supplements because there are no detrimental effects when those are supplemented in piglet feed. *Lactobacillus, Streptococcus, Bacillus, Aspergillus* and *Saccharomyces* are not only typical probiotics for animal feed but also commonly used microorganisms in life of human beings. Live probiotics are accustomed food supplement in human life as those are expected to have health benefits when people consumed or applied to human body. In 2006, antibiotics are banned by various countries in EU, animal feed industry needed to find since antibiotics are banned completely. Zinc oxide and probiotics are well verified feed additives for preventing diarrhea and promoting growth performance in young animals, respectively. Consequently, a study was prepared to figure out if there is a synergistic effect when zinc oxide and probiotics are supplemented in diet of piglet. In the feeding trial, a total of 300 crossbred piglets [(Yorkshire × Landrace) × Duroc], weaned at 28 ± 2 days of age with an average body weight (BW) of 6.67 ± 0.872 kg were allotted to 6 treatments and 5 replicates with 10 pigs per pen in a randomized complete block (RCB) design. Treatments were 1) NL: corn-soybean

meal base feed + probiotics 0.01%, 2) NM: basal feed + probiotics 0.05%, 3) NH: basal feed + probiotics 0.1%, 4) ZL: basal feed + probiotics 0.01% + zinc oxide, 5) ZM: basal feed + probiotics 0.05% + zinc oxide, 6) ZH: basal feed + probiotics 0.1% + zinc oxide. The amount of zinc oxide added in the phase I was 0.25% and the phase II was 0.025%. All other nutrients are met or exceeded requirements of NRC (2012). As a result of the entire 6-week experiment, the growth performance of weaned piglets that consumed zinc oxide was significantly higher in BW compared to those not ingested at week 3 and week 6 ($P<0.01$). In the case of average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F ratio) were significantly higher in Phase I and the entire period ($P<0.01$) when zinc oxide was supplemented treatments. Average daily feed intake (ADFI) was also significantly higher in the phase I and the whole period compared to the treatment group NL, NM and NH without the addition of zinc oxide treatment group ZL, ZM and ZH ($P<0.01$). Result of quantitative experiment in branching microorganisms, there was a significant difference in coliform count (CC) and yeast & mold (YM). In CC, NH with 0.1% of probiotics without addition of zinc oxide was extremely lower compared to other treatments ($P<0.01$). In the case of YM, ZM added with zinc oxide and 0.05% of probiotics was significantly higher than that of other treatments ($P<0.05$). In the case of Zinc-Quantitative experiment, *E. coli*/coliform count (EC) and yeast & mold (YM) were significantly higher and lactic acid bacteria count (LAB) was significantly lower in the treatment with zinc oxide ($P<0.05$). The diarrhea index was significantly lower in both the 3 weeks and 6 weeks when the treatment group ZL, ZM and ZH with zinc oxide added and the

treatment group NL, NM and NH without the addition of zinc oxide were significantly lower ($P<0.01$). There was no significant difference in immune response in the case of IgA. However, the level of Ig A was higher in the treatment group with probiotics than in the treatment group with zinc oxide. Consequently, Zinc oxide had a positive effect on growth performance and diarrhea index, but probiotics had a positive effect on IgA and intestinal microbes.

Key words: Probiotics, Zinc oxide, Weaning pig, Growth performance, Microorganisms, Diarrhea index, Fecal microflora

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III. Experiment

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List of Abbreviations

AC	:	Aerobic count
ADG	:	Average daily gain
ADFI	:	Average daily feed intake
BW	:	Body weight
CC	:	Coliform count
CRD	:	Completely randomized design
EC	:	*E. coli*/coliform count
LAB	:	Lactic acid bacteria count
ME	:	Metabolic energy
NRC	:	National Research Council
SAS	:	Statistical analysis system
SBM	:	Soybean meal
SEM	:	Standard error of the mean
YM	:	Yeast and mold count

I. Introduction

Weaning stress is the most important problem in piglet growth. After weaning, piglets are subjected to a lot of stress and must undergo environmental, nutritional, social and biological changes. These changes reduce piglets' feed intake, lose weight, and cause severe diarrhea. As a result, after weaning, the disease is prevalent and the mortality rate increases.

In the past, antibiotics were used to prevent diarrhea and disease caused by weaning stress. However, in recent years, the use of antibiotics has been banned not only in Korea but also in the world. After antibiotics were banned, zinc was added to prevent weaning stress. Essentially, 0-2000ppm was used, and 2000-4000ppm was added pharmacologically into piglet feed (Burch, 2014). However, the risk of excessive addition of zinc in pig feed is drawing attention worldwide. It is reported that excessive addition of zinc induces resistance to zinc and antibiotics, and the most prominent factor is environmental pollution. Therefore, not only the Korean government but also other countries around the world are planning to enforce strong sanctions against the addition of zinc in piglet feed.

In this situation, probiotics are attracting attention as a substitute for zinc. Probiotics have been widely used as substitutes for antibiotics in the past, but many studies have to be conducted because the types and contents are different. Therefore, this study was conducted to evaluate effect of zinc oxide and probiotics

on growth performance, blood profiles, immune response, diarrhea index and fecal microflora in weaning pigs.

II. Review of Literature

1. Introduction

The need for zinc has been revived since the 1950s. Zinc is an essential element for growth performance and pig health, and it has a number of functions in the body. Over time, it has been found that zinc is effective in preventing diarrhea caused by weaning stress.

As a result, 2500-3000 ppm, which is much higher than the NRC (2012) recommended amount of 80-100 ppm and the Korean feed management law recommended amount, of 120 ppm, is added to piglet feed. With this excessive use, environmental problems such as soil pollution and water pollution caused by zinc are becoming a problem. In a situation where antibiotics used to prevent weaning stress from piglets were first banned, and zinc is expected to be banned in the future, probiotics are emerging as the most important alternative.

Probiotics are used to prevent severe diarrhea, immunity and mortality caused by weaning stress in piglets. furthermore, the probiotics have effects of improving growth performance and improving the intestinal microflora in addition to the diarrhea preventing effect. As a result, various studies are being conducted to effectively replace zinc with probiotics.

2. Zinc

2.1 Characteristics and functions of zinc

Trace minerals such as zinc are essential nutrients required for various metabolic functions such as growth, development, reproduction and immunity. It is essential for the action of about 300 enzymes, and is also required for cell differentiation and DNA protein synthesis (Bhowmik et al. 2010). In addition, zinc is not stored in the body, so continuous supply in the form of feed is required (Lonnerdal, 2000). This is especially important for pigs, and the main cause is the phosphatase in pig feed. This is because phosphatase combines with Zn to form insoluble mineral chelates that are not absorbed in the intestine. In addition, the presence of higher amounts of other inorganic elements such as Fe, Cu and Ca blocks the absorption of Zn in the intestine (Krebs 2000).

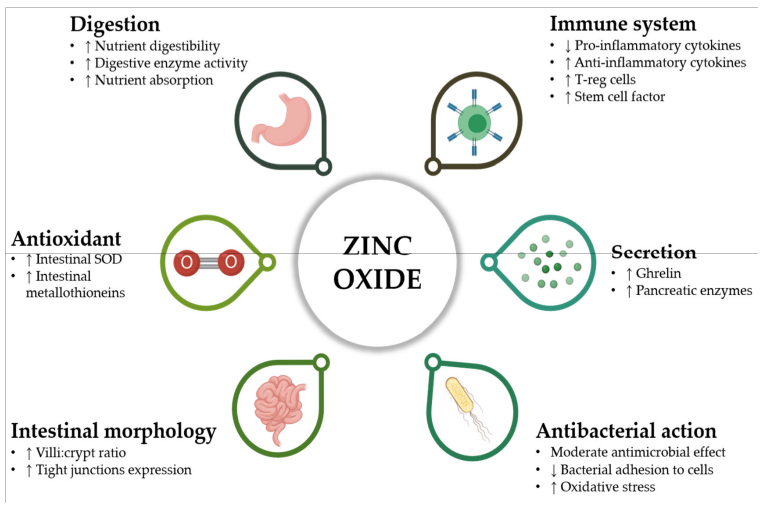

Figure 1. Beneficial effects and mechanisms of action of zinc oxide in post-weaning piglets (Bonetti et al., 2021)

Role of zinc pharmacological effect

Recent studies have reported that a high content of zinc in feed has a positive role in the general activity of animals as well as the immune system. Hahn and Baker (1993), Carlson et al. (1999) and Hill et al. (2000) showed that feeding 3,000 ppm zinc added as zinc oxide improved the growth and health of piglets. Long-term studies have demonstrated the pharmacological role of zinc as a feed additive for pigs. Currently, it is added to the feed of weaned pigs in many countries. In particular, the zinc oxide form is effective and is the most widely used.

Role of zinc on the gastrointestinal tract

Zinc has been implicated in maintaining gastrointestinal function, from the taste buds of the tongue to intestinal villus and crypt function (Berger, 2002). Studies have shown that gustin, an enzyme essential for taste bud development and function, is associated with the metabolism of zinc (Henkin et al. 1999). Zinc maintains the diversity of the gut microbiota (Katouli et al., 1999) and helps reduce susceptibility to *E. coli* infection in pigs (Mores et al., 1998). Further studies reported that supplementation with a pharmacological dose of Zn as zinc oxide acts as an antibacterial agent (Cromwell, 2001) and improves gastrointestinal function by increasing the mucosal thickness, villi height and width of the small intestine (Li et al., 2001). It was also observed that high dietary zinc (2500 ppm) increase the activity of enzymes viz-amylase, carboxypeptidase A, chymotrypsin, trypsin and lipase in the pancreatic tissue of pigs.

Role of Zn on enzyme activity

It has been found that zinc directly or indirectly affects about 300 enzymes. Especially, the role of Zn on serum alkaline phosphatase (ALP), glutatamate oxaloacetatetransaminase (GOT), glutamate pyruvate transaminase (GPT), erythrocyte Cu Znsuperoxide dismutase (Cu/Zn SOD) was studied detail in Zn deficient and supplemented pigs (Borah et al, 2012). According to previous studies, the activities of ALP, GOT, and GPT in serum changed with zinc deficiency, and all species, including pigs, recovered to normal levels when sufficient zinc was supplied (Vergnes et al., 1990; Petkevicius et al., 2003; Sidhu et al., 2005).

In addition, Cu / Zn SOD enzyme plays an essential role in vital activity, and it affects reactive oxygen species (ROS) such as superoxide radicals (O2-), hydroxyl radicals (OH-) and hydrogen peroxide (H2O2) and mammalian tissues. Excessive generation of reactive oxygen species can cause detrimental changes, such as lipid peroxidation, DNA breakage, protein degradation, and enzyme inactivation (Dennery, 2007) that lead to cellular death. Therefore, these free radicals must be eliminated from the body. Among the numerous enzymes, the most effective enzyme that catalyzes free radical removal is Cu/Zn SOD. As the name suggests, you can see the importance of zinc's role in enzymes.

Role of Zn on metabolic hormone (tri-iodothyronine and thyroxin) activity

In order to maintain a normal thyroid state, trace minerals for the synthesis and metabolism of thyroid hormones are essential. The role of zinc in thyroid metabolism has been studied by many scholars, but the results are somewhat different (Arthur and Beckett 1999; Baltaci et al., 2004).

However, according to Borah et al., 2014 study, it was confirmed that the concentration of tri-iodothyronine and thyroxin, a kind of thyroid hormone, was high or low depending on whether or not zinc was added to pig feed. they concluded that that might be due to the fact that Zn is associated with maintaining the normal physiological status of the thyroid gland (Hartoma et al. 1979) and thyroid follicles (Gupta et al. 1997).

Role of Zn on growth:

When zinc is added to pig feed, it activates the action of enzymes and is involved in cell division and proliferation, which has a positive effect on growth performance (Mac Donald, 2000). Conversely, if zinc is deficient, growth will be stunted. Growth disorders due to zinc deficiency started in hypogeusia and were alleviated with zinc supplementation (Tomita, 1977; Tomoko et al., 2001).

Neto et al. (1995) divided the influence of zinc on tissue growth and development into the following three types. First, it sensitizes taste and smell, and affects appetite and food intake. As shown in figrure 2, the decrease in appetite is not a simple decrease in feed intake, but negatively affects growth by blocking hormone secretion and inhibiting signal transduction. Zinc deficiency reduces feed intake in rats by up to 30%. They also avoid carbohydrates and look for proteins and fats (Kennedy et al., 1998). Zinc deficiency also impairs taste. Taste is mediated through gustin a salivary zinc-dependent polypeptide, and zinc deficiency results in lower salivary zinc concentrations, making it difficult to taste and decreased appetite. Second, it participates in the action of hormones. It promotes

the synthesis and secretion of growth hormone, and under the influence of growth hormone, somatomedin-C is produced and activated. In addition to several of these functions, zinc also interacts with other hormones involved in bone growth, such as testosterone, thyroid hormone, insulin, and vitamin D3. Finally, it acts on DNA and RNA synthesis stimulation and affects Cell replication and differentiation of chondrocytes, osteoblasts and fibroblasts. Cell transcription culminate in the synthesis of somatomedin-C (liver), alkaline phosphatase, collagen and osteocalcin (bone). It also affects protein, carbohydrate and lipid metabolism, which is closely related to smell, taste, appetite, and mechanisms of food consumption and utilization based on these various factors, zinc plays a role in pig growth as well as in the formation of bones and proteins.

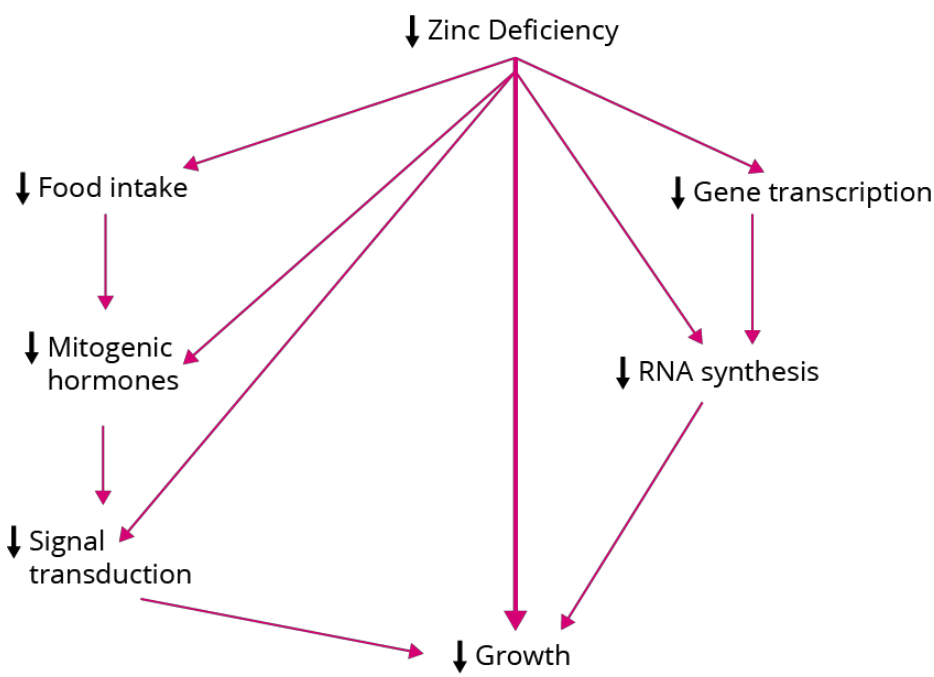

Figure 2. Effects of zinc deficiency on metabolic processes associated with growth (Macdonald, 2000).

Role of Zn on pig reproduction

For economic animals, reproduction is important. Therefore, efforts should be made to maintain a high reproductive capacity. This is true for both males and females, appropriate age, weight, etc. should be taken into account.

Sow is a polyoestrous breeder and the normal length of an oestrous cycle is 21 days. The oestruous cycle can be divided into a follicular phase, during which follicles grow and mature in the ovary, and a luteal phase, following ovulation and characterised by development of corpora lutea (kalita et al., 2017). Zinc has been shown to be associated with sexual maturity, estrus induction and estrus cycle in sows (Borah et al., 2014). It is also associated with the size and number of pups at parturition (Borah et al., 2014). Zinc plays an important role in the recovery and maintenance of the endometrium after delivery and helps restore estrus (Green et al., 1998). It has also been shown that levels of follicle-stimulating hormone and luteinizing hormone are low when zinc is deficient (Boland, 2003).

For males, zinc is important for reproduction. If zinc is deficient, the development of Leydig cells may be delayed and the response to LH as well as testicular steroid production may be reduced. Therefore, zinc is very important for spermatogenesis.

Seminal plasma is important for sperm motility (Rodriquez-Martinez et al., 1990), protects the membranes of sperm cells, and helps maintain fertility while in the male body (Harrison et al., 1978) . Zinc is one of the substances that plays an important role in this

seminal plasma (Hamamah and Gatti 1998). In addition, zinc-binding proteins in semen plasma allow sperm to be protected from cold conditions and remain active (Mogielnicka-Brzozowska et al., 2011). In addition, zinc directly affects the process of sperm formation. It aids in sperm formation, maintains motility (Wroblewski et al., 2003), acts as an antioxidant (Gavellaand Lipovac 1998), and prevents deterioration during non-fertilization (Suruki et al., 1995).

Role of Zn on immune system

Zinc deficiency leads to reduced immunity and loss of T cell function in animals. Zinc plays a very important role in maintaining the immune system, and pigs deficient in zinc are susceptible to various diseases.

Zinc affects different immune systems in a variety of ways, from the skin barrier to the regulation of genes within lymphocytes. Zinc is crucial for normal development and function of cells mediating non-specific immunity such as neutrophils and natural killer cells. Deficiency of zinc in pig feed adversely affects the development of acquired immunity by blocking both external growth and specific functions of T lymphocytes, such as Th1 cytokine production and activation of B lymphocytes. In addition, B lymphocyte development and antibody production, especially immunoglobulin G, are also impaired. Macrophages are also most adversely affected by zinc deficiency. Zinc deficiency can interfere with the regulation of intracellular killing, cytokine production and phagocytosis. The effects of zinc on these key immunologic mediators are rooted in the myriad roles for zinc in basic cellular functions such as DNA replication, RNA transcription,

cell division, and cell activation (Shankar and Prasad,1998).

In humans as well as in complex animals such as pigs, thymic atrophy, lymphopenia, and damaged cell- and antibody-mediated reactions that lead to increased infection rates occur when zinc is deficient. As the deficiency worsens, the immune system is reset. Chronic production of glucocorticoids that accelerate apoptosis begins between pre-B and T cells. This in turn reduces lymphopoiesis and causes atrophy of the thymus. On the other hand, myelopoiesis is preserved, thereby providing protection for the first line of immune defense or innate immunity. Changes in gene expression for cytokines, DNA repair enzymes, zinc transporters, signaling molecules, etc., suggest that cells of the immune system are attempting to adapt to the stress of suboptimal zinc.

The thymus plays an important role in the production of T-cells. Zinc deficiency causes changes in the immune system such as: It induces lymphocytic atrophy and reduces the body's response to numerous T-dependent antigens (Fraker et al., 1977; Chandra, 1985). Reduces the number of IgM and IgG plaque-forming cells per spleen in response to immunization with sheep red blood cells. Zinc deficiency interferes with T-cell helper function, resulting in significant defects in humoral immune capacity (Moulder and Steward, 1989). Eventually, it adversely affects the concentration of thymic hormone and decreases the thymus weight (Golden et al., 1977).

It was investigated that the immunity of pigs is decreased due to zinc deficiency. This affects young animals more adversely than adults. This is because not only the results of deficiency appear quickly in young animals when they are

malnourished, but there is no preventive mechanism in vivo because they have never experienced the disease (Beach et al., 1982). Therefore, zinc deficiency in young livestock should be considered important

The role of zinc on biological systems

Zinc is a structural component of numerous proteins, including enzymes and transcription factors in cellular signaling pathways. Zinc can regulate cell signal recognition, second messenger metabolism, and protein kinase and protein phosphatase activity. Zinc can modulate cellular signal recognition, second messenger metabolism, protein kinase, and protein phosphatase activities.

in addition to calcium, phosphorus, and magnesium, zinc is important for bone formation as zinc deficiency has been shown to reduce the size and strength of the femur. Parakeratosis, the thickening, hardening and cracking of skin is a common sign of zinc deprivation in all species. Poultry usually develops skin disease on the feet and feathers, whereas pigs develop skin disease all over the limbs. Zinc deficiency greatly retards the rate of healing of skin wounds in all species.

It is essential for cell proliferation and differentiation, especially for the regulation of DNA synthesis and mitosis (Miller et al. 1968). Zn plays an important role in maintaining genome stability, genetic expression, and regulation of apoptosis. Zn is an essential part of the DNA repair protein OGG1, which repairs oxidized guanine in DNA. Dysregulation leads to point mutations and down regulation in gene expression (Thomas et al., 2015).

2.2 Use in piglet feed

Pigs are usually weaned between 3-5 weeks. This is much shorter than the normal 17 weeks in the natural environment (Jensen and Recén, 1989). weaning is the biggest stress in a pig's life. This is because a number of external and internal stressors are complexly occurring.

Weaning pigs undergo social changes in which they are separated from their mothers and have to live in groups with several pigs (Pluske et al., 1997). In addition, the feed system and breeding environment change rapidly (Weary et al., 2008), the microorganisms are not yet perfect (Wang et al., 2013), the ability to regulate body temperature is low (Le Dividich and Herpin, 1994), and the digestion ability is also fall (Lallès et al., 2007a). As a result, serious problems appear in the growth and survival of pigs (Lallès et al., 2007b). At this point, various pathogens such as Salmonella *E. coli* and diseases such as diarrhea threaten piglets (Pluske e tal., 1997; Fouhse et al., 2016). Altogether, the process is known as a post-weaning syndrome and has been extensively studied and reviewed (Pluske etal., 1997; Lallès etal., 2007a; Heo etal., 2013). Antibiotics were used exclusively to prevent post-weaning syndrome, but are now banned, and zinc oxide is the most used.

Zinc exists in various compounds, and various studies have been conducted to reduce post-weaning stress and diarrhea in pigs. The most used zinc source along with zinc oxide is zinc sulfate. Sulfate has some effect on the prevention of diarrhea by intestinal pathogens (Surjawidjaja et al., 2004), but the absorption rate is too high. A high absorption rate of zinc shortens the intestinal residence time and interferes with its antibacterial effect. In addition, there are other zinc compounds

such as zinc chloride and tetrabasic zinc chloride, but they have lower antidiarrheal and antibacterial effects than zinc oxide.

Zinc oxide is effective in preventing disease, enhancing immunity, and preventing diarrhea. In order to prevent side effects due to weaning stress, zinc oxide should be added in excess for pharmacological effects, not the usual amount.

Through various studies, it has been found that there is a pharmacological effect when 2000-3000 ppm of Zinc in pig feed is added (Hill et al., 2000; Calson et al., 1999; Hahn and Baker, 1993). Katouli et al. (1999) found that 2,500 ppm zinc in diets of weanling pigs maintain the stability of intestinal microflora and diversity of the coliforms for the first 2-weeks after weaning. Carlson et al. (1998) reported that feeding 3,000 ppm zinc as zinc oxide produced deeper crypts and greater total thickness in the duodenum. However, Europe is leading, and countries are regulating zinc oxide. Due to environmental pollution, it will be banned from use in Europe as early as 2022. Therefore, new methods to replace zinc oxide and prevent weaning stress and diarrhea should be founded.

3. Probiotics

3.1. Characteristics and functions of probiotics t

The most generally used definition for probiotics is the one proposed by the United Nations and World Health Organization Expert Panel in humans, as “live micro-organisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO 2001). Probiotics were first reported in 1906

through early observations of “beneficial bacteria” by Metchnikoff and Tissier, and for a long time have attracted attention among the scientific community, especially pig nutritionists. Metchnikoff drew attention to the relationship between dairy products consumed by Bulgarian farmers and longevity, and thought it might be an effect of beneficial bacteria present in the colon. In the future, he also sees probiotics as preventing many human diseases associated with colorectal disease. To date, there are countless types of probiotics that have been discovered and used directly in industry, and related research is still ongoing.

Since various microbial strains have different effects, they can be used as raw materials for probiotics. However, depending on the characteristics of the host, it may or may not provide a positive effect (Weichselbaum, 2009). The most used probiotics are lactic acid bacteria such as *Lactobacillus, Bifidobacterium* and *Streptococcus*. These species have excellent stability in gastric and bile acids, and excellent intestinal activity and pathogen resistance (Verdenelli et al., 2009). The microorganisms commonly used as probiotics for livestock are as follows. *Lactobacillus- acidophilus, casei, brevis, fermentum, gallinarum, plantarum, gasseri, johnnsonii, reuteri, salivaris, Bifidobacterium- bifidum, lactis, Saccharomyces- cerevisiae, boulardii, Aspergillus- tiloryzae, Enteris- coccus, boulardii, Aspergillus- tiloryzae, Bacocillus- faecium and Pediococcus- pentosaceus* (Ohashi and Ushida, 2009).

The characteristics of probiotics can be broadly divided into four important properties. First of all, probiotics must be stable in gastric acid and digestive

environment for intestinal activation and metabolism. Second, it promotes health either directly by stimulating the host immune response or indirectly by lowering the influence of pathogenic bacteria. Third, industrial applicability is essential. Finally, stability.

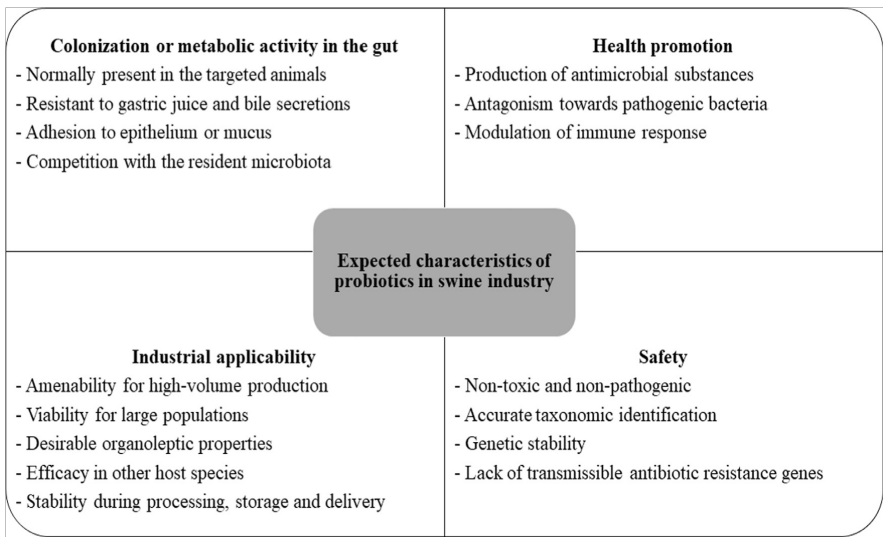

Figure 3. Expected characteristics of probiotics (Barba-vidal et al., 2019)

Role of probiotics on gut bacteria and health

The benefits of feeding probiotics are related to the bacteria in your gut. In humans, the number of bacteria is ten times greater than the cells that make up the host body. Various types of bacteria, yeasts and viruses are present in the intestines of pigs as well as humans (Sears, 2005). Bacteria in the intestinal tract are also called another indispensable organ because they play a very important role (Kim et al., 2007; Klaenhammer et al., 2008).

Therefore, animals raised in the absence of bacteria show serious abnormalities in intestinal morphology and immune function after growth (Nanthakumar et al., 2003; Wagner, 2008). When intestinal bacteria are positively activated through probiotics, the metabolism of vitamins is smoothed, digestion is increased, and pathogens can be defeated. It is effective in maintaining the intestinal barrier and function and relieves the inflammatory response. In addition, it acts as a natural antibiotic to prevent disease (Madsen et al., 2001; Hooper et al., 2002; Ouwehand et al., 2002; Roselli et al., 2007).

Studies have reported a novel role in regulating fat storage. It promotes absorption of monosaccharides in the intestine and induces adipogenesis (Ba¨ckhed et al., 2004). This is very important for economic animals, and it was found by comparing the gut microbiome and energy metabolism function of obese and lean mice (Turnbaugh et al., 2006).

The formation of beneficial gut microbiota is important for newborn animals. This is because beneficial bacteria themselves not only prevent disease, but also recognize pathogens and form the immune system (Calder et al., 2006; Williams et al., 2006; Boirivant et al., 2008). It is very important to create such an early intestinal environment. This is because it is an important basis for the host to recognize what is dangerous. Based on this, the host responds to external and internal factors even after becoming an adult (Zoetendal et al., 2001; Favier et al., 2002). In fact, gut microbiota differs depending on the environment in which the host was raised (Mueller et al., 2006). Therefore, various beneficial

microorganisms should be fed or exposed to the host as soon as possible. This is also true for humans. The importance of early-life exposure on subsequent development of a rich, diverse microbiota is shown in studies comparing the microbiota of children normally delivered and those delivered by caesarean section; the latter children had markedly less complex microbiota (Gronlund et al., 1999; Biasucci et al., 2008).

There are opposing opinions. This is because there is basically no problem for newborn pigs to survive and grow even with the intestinal microbes they have been given in the natural environment. In addition, the essential microflora has already been mostly established at birth (Konstantinov et al., 2006; Thanantong et al., 2006). However, there are also opinions that a sufficient microflora cannot be obtained due to the nature of economic animals born in facilities that are regularly sterilized. This has indeed been shown in recent studies in pigs raised in different high v. low hygiene environments, which showed that such differences significantly affect not only intestinal microbiota composition but also the mucosal innate immune function in neonates, as well as in adult animals (Mulder et al., 2009; Inman et al., 2010). In the future, various studies should be conducted on the formation of beneficial bacteria in the intestine of newborn piglets, but for pigs, the formation of beneficial bacteria in the intestine through probiotics and their benefits are clear.

Role of probiotics on gastrointestinal tract

Probiotics have a positive effect on the digestion, absorption and secretion of enzymes in the pig intestine. When 28-day-old piglets were fed *Bacillus cereus* or *Enterococcus faecium*, slightly higher L-glutamine transport and increased ion secretion were observed in pigs (Lodemann et al., 2006; Lodemann et al. al., 2008). In addition, In the case of *Lactobacillus sp. PSC101*, it showed a positive effect on the production of enzymes such as amylase, lipase, phytase and protease. Study in germ-free mice using the organism *Bacteroides thetaiotaomicron* has shown that introduction of the bacteria is critical for induction of critical glycolytic enzymes in the enterocytes (Bry et al., 1996). Consequently, for immature animals such as piglets, the feeding of probiotics has an important role in activating host metabolism.

Role of probiotics on enterocyte stability

When *lactobacilli* and *enterocytes* were co-cultured, it was possible to protect the barrier membrane from destruction by pathogens. This appears to be a multi-factorial process involving both induction of mucus secretion from goblet cells (Mack et al., 1999; Caballero-Franco et al., 2007) and maintenance of the tight cell junctions between cells (Madsen et al., 2001; Roselli et al., 2007; Putaala et al., 2008). In addition to the mechanisms described, probiotics may provide defense against cells through induction of anti-inflammatory cytokines and reduction of pro-inflammatory cytokines. (O'Hara et al., 2006; Walsh et al., 2008; Wang et al.,

2009). In addition, Cytokines promoted by probiotics also maintain the stability of the enterocyte barrier (Roselli et al., 2007).

Role of probiotics on immune system

Probiotics play an important role in the immune system. Various types of antimicrobial mechanisms exist in the gut. Of particular interest is defensin. Defensin is a pore-forming antimicrobial peptide produced by Paneth cells and other cells including neutrophils and macrophages. These molecules act as antimicrobials by directly inhibiting pathogen growth, as well as potentiating branches of the innate, humoral and cell-mediated immune system (Linde et al., 2008). Defensin induction seems to be a common and important mechanism of probiotic treatment (Mondel et al., 2009). Studies have shown that VSL#3 (four *Lactobacillus* species, three *Bifidobacterium* species and one *Streptococcus*), a frequently used probiotic combination, is a potent inducer of b-defensin. The mechanism appears to be via nuclear factor (NF)-kB and activator protein-1 (AP1) intermediates, which is interesting as probiotics are intuitively regarded as being anti-inflammatory (Schlee et al., 2008).

In addition, probiotics increased levels of immunoglobulin measured in the serum and feces of pigs, and increased production of specific antibodies against Salmonella when fed with E. faecium (Szabo et al., 2009). When pregnant sows were fed *B. cereus* or *E. faecium*, it was confirmed that the serum IgG of weaned

pigs was significantly reduced (Scharek). et al., 2007). In addition, IgA levels were increased in the group administered with B. cereus compared to other treatments. IgA is important for immunity because it is hypothesized that it can reduce inflammation and eliminate harmful factors that can lead to disease in advance. In addition, blood lymphocytes increased in weaned pigs fed *L. fermentum*, and the pro-inflammatory cytokines IFN-g and TNF-a were increased in the ileum. In addition, rotavirus-induced inflammation was restricted in the intestines of gnotobiotic pigs fed with *L. acidophilus* and *L. reuteri* (Zhang et al., 2008).

Role of probiotics on biological systems

Numerous bacteria are present in the intestines of pigs and vertebrates. There are some types of bacteria that are not beneficial to the pig's immunity, and they use energy to fight them. In this case, there is a possibility of inhibiting the growth of pigs from a nutritional point of view. The addition of probiotics can be an important method to solve diseases and growth stagnation that may occur at this time.

Studies have shown that feeding probiotics to weaned pigs infected with *E. coli* F4 resulted in an increase in average daily body weight as well as treatment of pathogens (Konstantinov et al., 2008). According to Cheeson (1994), the ratio of amino acids available for metabolism changes according to changes in the intestinal bacterial flora of pigs. The amino acids required for pig growth are not

only absorbed through feed. It is also provided by the gut microbiota, and studies have shown that it provides from 1 to as much as 20% lysine (Metges, 2000).

3.2. Use in piglet feed

Since the use of antibiotics was banned to reduce weaning stress, the zinc content in piglet feed increased. As well as antibiotics, zinc also has long-term negative effects on the environment and pig growth. For this reason, probiotics in piglet feed are used.

The pig placenta does not transport maternal immunoglobulin and therefore newborn piglets acquire maternal immunoglobulin from colostrum during the first 24 to 48 h of life. The mucosal immune system and more especially the T-cell component of the intestinal mucosa of the newborn piglet is poorly developed at the time of birth and during the first few weeks of life, it undergoes a rapid period of expansion and specialization (Lalles et al., 2007). Therefore, piglets who do not consume enough colostrum are vulnerable to immunity before and after weaning, and immunity must be reinforced. Various studies have shown that the addition of probiotics in pig feed has an effect on immunity enhancement (Erickson and Hubbard, 2000; Delcenserie et al., 2008;). Probiotics have also been shown to be effective in preventing diseases and pathogens in pigs (Bhandari et al., 2008; Cheikhyoussef et al., 2008). It also stabilized the intestinal microflora of piglets (Simmering and Blaut, 2001), stabilized the intestinal tract, and was effective in enhancing immunity (Zhang et al., 2010; Klaenhammer et al., 2012; Prieto et al., 2014; Zacarías et al., 2014).

Probiotics also have a positive effect on the growth performance of weaned pigs (Datt et al., 2011). The initial weight of weaned piglets is directly related to farm profitability as an economic point of view (Campbell, 1997). An increase in pig weight at weaning with one kg will result in a pig which reaches slaughter weight at least 10 days faster (Cole and Cole, 2001) and accepted that average daily gain during the first week post-weaning has a major impact on subsequent growth performance (Tokach et al., 1992).

The gut microbiome is directly related to the health of pigs. The - major intestinal flora of pig is *Lactobacilli, Saccharomyces, Bifidobacteria, Streptococci, Bacteriodes, Clostridium perfringes* and *E. coli*, this microflora changes with age. It is important to balance the microbes, but there are situations in which *E. coli* dominates when piglets are weaned. Probiotics increased the beneficial bacteria *Lactobacilli* and decreased the number of *E. coli* (Huang et al., 2004).

After weaning, the morphology of the pig intestine changes due to the consumption of hard solid feed (Hampson, 1986). The length of Villus decreases, and the depth of crypt increases. The shape of the intestine should be supplemented because it directly affects the growth and health of pigs. According to a study, when probiotics were fed into piglets feed, intestinal morphology was restored (Gebert et al., 2011; Bontepmo et al., 2006).

There are a few things to keep in mind when feeding probiotics to piglets. Studies have shown that some probiotics cause negative effects when piglets are already diseased (Barba-vidal et al., 2018). Therefore, depending on the raw material of the probiotic, it is necessary to distinguish whether it is used for

prevention or treatment.

More than 80% of studies among the vast amount of data showed that the use of probiotics in piglet diets had a positive effect (Barba-vidal et al., 2018). However, it is not easy to give neutral or negative results due to the nature of probiotics that are influenced by industry (Fanelli, 2012). Therefore, continuous, accurate and objective research is necessary.

4. Environmental pollution caused by zinc

4.1. Environmental pollution

In the past antibiotic use period, the problem of antibiotic residue in soil and water has been pointed out. However, the use of antibiotics has been banned entirely in many countries, and another problem has arisen. It is a heavy metal contamination problem that is added to promote growth and prevent diarrhea. The environmental pollution of zinc oxide also belongs to this category.

Zinc is toxic because it is a heavy metal, so a certain proportion is absorbed and physiologically excess zinc is excreted. When zinc oxide is ingested, only 14% is absorbed into the body, and 86% is excreted from the body. Zinc excreted in pig manure is a major source of zinc entering the environment (Lopez et al., 2000). According to Bak et al., 2015 study, discharge of Zn-rich porcine slurries during the 1986-2014 period increased the Zn concentration in the soil by 2-5%, with an average increase of 24% during the sampling period (1998-2014). This has been proven through various studies, and copper and zinc added to promote growth and

prevent diarrhea have a direct effect on soil and water pollution (Poulsen, 1995; Katouli et al., 1999; Hill et al., 2000).

Soil pollution and water pollution through zinc do not simply mean each geographic pollution. Zinc that is continuously released is organically linked to each other through contamination of soil, leachate, groundwater, and surface water. In addition, heavy metals accumulate in plants and grains grown on the basis of contaminated soil and water. It spreads to various animal species through the ecological chain and affects even humans (Formentini et al., 2017; Zhang et al., 2018a, b).

4.2. Zinc regulation in global

The world, especially Europe, is actively regulating the addition of zinc oxide in weaning pig feed. As a result of the 2017 vote, the addition of more than 150 ppm of zinc oxide in weaning pig feed will be banned in Europe from June 2022. After the UK withdrew from the EU, there was a movement to register and use zinc oxide-added products through the UK, but this attempt failed because the Netherlands and France strongly opposed it.

Other countries, including the United States, have not yet initiated strong sanctions against zinc oxide. However, many countries agree on the environmental pollution caused by zinc oxide, and a consensus on sanctions is being formed. However, a premature ban on zinc oxide may have more serious side effects. Take South Africa as an example. South Africa banned the addition of zinc oxide to weaning pig feed, which led to overuse of antibiotics and serious intestinal

problems in pigs. Therefore, each country should think carefully about the regulation of zinc oxide.

4.3. Zinc regulation in Korea

Korea is also regulating the addition of zinc in the weaning pig feed. But it is not strict than other countries. Korea is based on the addition of zinc in weaning pig feed by 120 ppm or less. However, it allows to be 2500 ppm for pharmacological effects. In the case of Korean feed, it is generally added to 2000 ppm to prevent diarrhea. However, recently, Korea's feeding companies are focusing on zinc issues, and they are promoting zinc reduction feeds.

The government of Korea is not only regulating the content of zinc in the feed, but also on the liquefied manure and compost. liquefied manure must be less than 170 ppm, and compost must be less than 1200 ppm.

5. Probiotics as a zinc replacement

5.1. Alternative feed additives for Zinc

Zinc has a great effect on the growth of weaned pigs and disease prevention, but its use is banned or reduced internationally because of environmental pollution and various other side effects. There are several reasons for reducing the use of zinc. First, pigs and microorganisms are increasingly resistant to zinc at the species level (Stark et. al., 2013). In addition, it was found that high doses of zinc were also associated with antibiotic resistance (Stark et. al., 2013). In addition, it has

been reported that the use of zinc increases resistance to *E. coli* (Bednor, 2013) and Tetracycline (Vahjen 2015 and 2017). Also, because it is a heavy metal, it may act harmful to the body. These are summarized in figure 4.

Various methods exist to replace zinc oxide and prevent diarrhea. There are ways to reduce the intake of crude protein, add insoluble fiber, or limit the intake of iron. However, feeding feed additives is the most preferred and effective. Feed additives for replacing zincs are usually related to animal intestinal health. Probiotics is the most attractive, and products using organic acids, herbal extracts, yeast and seaweed are also considered as an alternative to zinc. In addition, a method is being studied to change the form of zinc to reduce the content of zinc in the weaning pig feed. As an example, zinc is produced in a nanoparticle size, and there is a method of increasing absorption by a porous product that punched with a hole in zinc particles. However, this is a pricing problem, and its effect seems to be not large compared to zinc oxide.

Figure 4. Risks related to pharmacological zinc oxide utilization in post-weaning piglets (Bonetti et al., 2021)

5.2. Effectiveness of probiotics as a zinc replacement

When probiotics are fed to weaning pig, it is sufficient as a substitute for zinc oxide because it contributes to intestinal health and stabilization, enhances immunity, and reduces diarrhea.

Several studies have demonstrated the benefits of supplementing with various probiotics to prevent damage by *E. coli* F4, a major causative agent of weaning stress-induced disease. The most commonly used probiotics in general products are lactic acid bacteria and yeast. Roselli et al. Feeding probiotics such as *Lactobacillus sobrius, Lactobacillus rhamnosus* and *Bifidobacterium* animalis to feed has been shown to prevent *E. coli* F4 adhesion and damage to tight junctions to intestinal epithelial cells with significant modulation of inflammatory cytokines (Roselli et al., 2006; Roselli et al., 2007). Feeding *Lactobacillus rhamnosus* to weaning pigs infected with *E. coli* F4 resulted in positive changes in gut microbiota and inflammatory cytokines, resulting in a significant reduction in diarrhea (Zhang et al., 2010). In addition, it was reported that when *Saccharomyces cerevisiae* was fed to weaning pigs through feed, diarrhea was reduced and it was reported that it had a positive effect on growth performance (Nordeste et al., 2017). Overall, probiotics, which are effective in reducing diarrhea in weaning pigs and enhancing immunity, and showing a positive effect on growth performance, have the potential to replace zinc oxide.

Ⅲ. Effect of zinc oxide and probiotics on growth performance, immune response, diarrhea index and fecal microflora in weaning pigs

ABSTRACT: This experiment was conducted to investigate the effect of the addition of zinc oxide and probiotics in feed on weaning pig. In the feeding trial, a total of 300 crossbred piglets [(Yorkshire × Landrace) × Duroc], weaned at 28 ± 2 days of age with an average body weight (BW) of 6.67 ± 0.872 kg were allotted to 6 treatments and 5 replicates with 10 pigs per in a randomized complete block (RCB) design. Treatments were 1) NL: corn-soybean meal base feed + probiotic 0.01%, 2) NM: basal feed + probiotics 0.05%, 3) NH: basal feed + probiotics 0.1%, 4) ZL: basal feed + Probiotic 0.01% + zinc oxide, 5) ZM: basal feed + Probiotic 0.05% + Zinc oxide, 6) ZH: basal feed + Probiotic 0.1% + Zinc oxide. The amount of zinc oxide added in the phase I was 0.25% and the phase II was 0.025%. All nutrient requirements were based on NRC 1998. However, since the CP was considered to be high, the required amount was set by multiplying the total nitrogen of NRC 2012 by 6.25. As a result of the entire 6-week experiment, the growth performance of weaned piglets that consumed zinc oxide was significantly higher in BW compared to those not ingested at week 3 and week 6 ($P<0.01$). In the case of Average daily gain (ADG), compared to treatment group NL, NM and NH without zinc oxide addition, treatment group ZL, ZM and ZH with zinc oxide was significantly higher in Phase I and the entire period ($P<0.01$). Average daily

feed intake (ADFI) was also significantly higher in the phase I and the whole period compared to the treatment group NL, NM and NH without the addition of zinc oxide treatment group ZL, ZM and ZH ($P<0.01$). Gain to feed ratio (G:F ratio) of treatment group ZL, ZM and ZH with zinc oxide added was significantly higher in Phase I and the entire period when compared with treatment group NL, NM and NH without zinc oxide addition ($P<0.01$). Result of quantitative experiment in branching microorganisms, there was a significant difference in coliform count (CC) and yeast & mold (YM). In CC, NH with 0.1% of probiotics without addition of zinc oxide was extremely significantly lower compared to other treatments ($P<0.01$). In the case of YM, ZM added with zinc oxide and 0.05% of probiotics was significantly higher than that of other treatments ($P<0.05$). In the case of Zinc-Quantitative experiment, *E. coli*/coliform count (EC) and yeast & mold (YM) were significantly higher and lactic acid bacteria count (LAB) was significantly lower in the treatment with zinc oxide ($P<0.05$). There was no significant difference in immune response in the case of IgA. However, the level of Ig A was higher in the treatment group with probiotics than in the treatment group with zinc oxide. Consequently, Zinc oxide had a positive effect on growth performance and diarrhea index, but probiotics had a positive effect on IgA and intestinal microbes

Key words: Probiotics, Zinc oxide, Weaning pig, Growth performance, Microorganisms, Diarrhea index, Blood profiles

Introduction

It is diarrhea caused by weaning stress that is directly linked to the mortality of weaning pig. Weaning Stress is the most extreme stress during the pig's growth stage as well as during the piglet period. The causes of weaning stress are diverse, including immature gut, change in feed form, change in pig farm environment, separation from sow, and incorporation into a new piglet group. Because of this stress, weaning pig reduce feed intake and energy absorption and adversely affect the intestinal epithelial structure (Pluske et al., 1996). In addition, the digestion and absorption of nutrients in the weaning piglet is reduced, creating an environment that is easy to proliferate intestinal pathogens (Spreeuwenberg et al., 2001; Boudry et al., 2004), and this structure is likely to cause diarrhea. Representative pathogens that cause piglet diarrhea after weaning are Salmonella and *E. coli*, which induce acute infections in the intestine and at the same time cause acute sepsis and enteritis, causing growth stasis such as diarrhea and death (Foley and Lynne, 2008).

In the past, antibiotics have been used to reduce mortality in weaned piglets, prevent diarrhea, and promote growth. However, in Korea, the addition of antibiotics in feed has been completely banned since July 2011. Currently, zinc oxide and probiotics are used as an antibiotic substitute and to prevent diarrhea in weaned piglets.

Zinc is an essential trace mineral for weaning pig and is widely used as a substitute for antibiotics (Slifierz et al., 2015). Depending on the amount of addition, as little as 0 – 2000 ppm or less, as a pharmacological level, up to 2000 –

4000 ppm is used (Burch, 2014). Zinc oxide in the feed for weaning pig is especially added to improve growth performance and prevent diarrhea (Hahn and Baker, 1993; Hill et al., 2001). In addition, the addition of zinc oxide aids in the development of intestinal villi of weaning pig and affects the health of the intestinal mucosa, which is beneficial for nutrient absorption. Li et al. (2006) confirmed positive changes in the length of villi and the composition of intestinal microbes when zinc oxide was added at 3000 mg/kg. Katouli et al. (1999) reported that when 2,500 mg/kg of zinc oxide was added, the intestinal microbial composition of weaned piglets was stabilized. Kim et al., (2012) reported that zinc oxide inhibits the proliferation and activation of intestinal mast cells, thereby reducing the release of inflammatory histamine and enhancing immunity.

Probiotics used as substitutes for antibiotics are 'living microbial feed additives that can have a beneficial effect on host animals by maintaining the balance of the host's intestinal microflora' (Fuller, 1989). Probiotics are also used to prevent growth stagnation, severe diarrhea, immunity enhancement, and mortality due to stress of weaning pig (Martin et al., 2009; Pieper et al., 2009; Szabo et al., 2009). According to Manner and Spieler (1997), it was reported that the addition of probiotics in the feed for weaning pig was effective in preventing diarrhea in weaned piglets. In addition, probiotics not only prevent diarrhea, but also have the effect of promoting growth and improving the intestinal microflora (Havenaar and Veld, 1992).

It has been reported that probiotics can replace zinc oxide, which is associated with environmental pollution, as well as improving the growth performance of weaning pig. According to Zong et al. (2018), the addition of probiotics, especially *Bacillus licheniformis* and *Clostridium butyricum*, in weaned piglet diets could replace zinc oxide in weaned piglet diets. According to Duc et al. (2004), it has been reported that *Bacillus licheniformis* enhances growth performance and immunity at the same level as zinc oxide. Zhang et al. (2014) reported that the addition of *Clostridium butyricum* in the feed for weaning pig had a positive effect on growth performance, immunity and intestinal microflora.

Until now, various prior studies have been conducted on the addition of probiotics in weaned pig feed as a substitute for zinc oxide, but there is no research on whether probiotics are effective as a substitute for zinc oxide in Korea.

Therefore, this study was conducted to determine the effect of zinc oxide and probiotics on growth performance, blood profiles, immune response, diarrhea index and fecal microflora in weaning pigs.

Materials and Methods

Experimental animals and diet

A total of 300 crossbred piglets [(Yorkshire × Landrace) × Duroc], weaned at 28 ± 2 days of age with an average body weight (BW) of 6.67 ± 0.872 kg were allotted to 6 treatments and 5 replicates with 10 pigs per in a randomized complete block (RCB) design. Treatments were 1) NL: corn-soybean meal base feed + probiotic 0.01%, 2) NM: basal feed + probiotics 0.05%, 3) NH: basal feed + probiotics 0.1%, 4) ZL: basal feed + Probiotic 0.01% + zinc oxide, 5) ZM: basal feed + Probiotic 0.05% + Zinc oxide, 6) ZH: basal feed + Probiotic 0.1% + Zinc oxide. The amount of zinc oxide added in the phase I was 0.25% and the phase II was 0.025%. All nutrient requirements were based on NRC (1998). However, since the CP was considered to be high, the required amount was set by multiplying the total nitrogen of NRC 2012 by 6.25. The formular and chemical of diets in all phase are presented in table 1, 2. The probiotic used in this experiment is a product obtained by solid fermentation of *Bacillus coagulance, Bacillus lichenformis*, and *Bacillus subtilis*, and a mixture of *Clostridium butyricum*. Probiotics were supplied in powder form from SynerBig, and the content was *Bacillus coagulance* 1×10^9 or more CFU/g, *Bacillus lichenformis* 5×10^8 or more CFU/g, *Bacillus subtilis* 1×10^9 or more CFU/g, *Clostridium butyricum* 1×10^8 or more CFU /g.

Growth trial

All pigs were housed in a wire-floored pen, equipped with a feeder and a nipple waterer and allowed *ad libitum* access to feed and water throughout the whole experimental period. The temperature was maintained at 30 ℃ in the first week and decreased 1 ℃ every week during last 4 weeks to be maintained 26 ℃ in the last week or trial. Body weight and feed intake were recorded beginning and end of each phase to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F ratio).

Blood profiles

Blood samples were collected anterior vena cava of 3 pigs per treatment, twice at 3 and 6 weeks for analyses of serum immunoglobulins A. All serum from blood samples were moved in serum tube (SST^{TM}II Advance, BD Vacutainer, Becton Dickinson, Plymouth, UK) and EDTA tube (BD Vacutainer K_2E, Becton Dickinson, Plymouth, UK). Individual sample was centrifuged at 3,000 rpm, 4℃ for 15 minutes (Eppendorf centrifuge 5810R, Hamburg, Germany) after clotting for 30 minutes at room temperature. The supernatant serum was separated to a microtube (Axygen, UnionCity, CA, USA) and stored at –20℃ for further Ig A analysis. Total serum IgA concentrations were determined by using ELISA starter kit (E101; Bethyl Laboratories Inc., USA) and microplate reader (Molecular pevices sunnyvale, CA, USA)

Evaluation of microbial population in fecal samples

1g of fecal sample was placed in sample bag (19 x 30 cm, 3M, St, Paul, MN, USA), adding 225 mL of 0.85% NaCl solution and using a homohenizer (JumboMix, Interscience, Saint Nom, France), homogenizing strongly for 2 min. After homogenous solution was serial diluted, cultivation was performed by selective medium of each microbial population. Latic acid bacteria was evaluated by Acid-MRS (pH 4.5) agar and cultured at 37 ℃ for 48 h. Aerobic bacteria was evaluated by petrifilm (Aerobic count plate, 3M, St, Paul, MN, USA) and cultured at 37 ℃ for 24 h. Yeast was evaluated by petrifilm (Yeast / Mold count plate, 3M, St, Paul, MN, USA) and cultured at 25 ℃ for 24 h. Small and having defined edges colonies were measured. Coliform was evaluated by petrifilm (Coliform count plate, 3M, St, Paul, MN, USA) and cultured at 37 ℃ for 24 h. The red colonies producing bubbles were measured. Bifidobacterium evaluated by Bifidobacterium Selective (BS) agar (KisanBio Co., Ltd. YangJaecheon-ro 31- 53 gil-11, SeoCho-Gu, Seoul, Korea) and cultured 37 ℃ in for 72 h. Amber color colonies were selected and transferred to MRS (added 0.5 % Lcystein·HCl) agar, identified by DNA sequencing.

Diarrhea index

The diarrhea index was measured twice a day after the start of the experiment at 8:00 am and 8:00 pm on the experimental piglets, and the diarrhea index was measured for all 10 weaning pigs in each pen during the entire experiment period. The diarrhea index was calculated as 0-10 by counting the number of diarrhea individuals out of 10 weaning pig in the pen. All diarrhea index measurements were performed by one person in charge to maintain objectivity as much as possible.

Statistical analysis

All collected data were analyzed as a completely randomized design using the General Linear Model (GLM) procedure in SAS (SAS Institute, 2004). Orthogonal polynomial contrasts were used to determine the linear and quadratic effects by increasing the vitamin premix levels in gestation for all measurements of sows and piglets. Individual sows and their litters were used as the experimental unit in physiological response, reproductive performance, blood profiles, milk composition. The differences among means were declared significant at $P<0.05$ and highly significant at $P<0.01$ and the determination of tendency for all analysis was $P\geq0.05$ and $P<0.10$. When the significance was declared, fisher's least significance difference (LSD) method was used to separate the means.

Results and Discussion

Growth performance

Table 3 shows the effect of addition of zinc oxide and probiotics in the feed for weaning pig on growth performance including body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F ratio).

As a result of the experiment, ZL, ZM, and ZH treatments with zinc oxide added were significantly higher in BW at 3 weeks and 6 weeks compared to other treatments ($P<0.01$). In the case of ADG, ZL, ZM, and ZH treatments with zinc oxide were significantly higher in Phase I and the entire period than NL, NM, and NH without the addition of zinc oxide ($P<0.01$). In the case of ADFI, the ZL, ZM, and ZH treatments with zinc oxide added were significantly higher in Phase I and the entire period compared to NL, NM, and NH without the addition of zinc oxide ($P<0.01$). In the case of G:F ratio, the ZL, ZM, and ZH treatments with zinc oxide were significantly higher in Phase I and the entire period compared to NL, NM, and NH without the addition of zinc oxide ($P<0.01$).

According to Hollis et al. (2005), it has been reported that the addition of 2,000 to 3,000 mg/kg of zinc oxide in the feed for weaning pig improves the growth performance of weaning pig. Despite the relatively low bioavailability of zinc oxide (Mavromichalis et al., 2000; Case and Carlson, 2002), this experiment showed a significant difference in BW at week 3 and week 6 compared to the

treatment without addition. However, unlike Phase I of weaning pig, there was no significant difference in ADG, ADFI, and G:F ratio of Phase II.

In this experiment, when probiotics were added in the feed of weaning pig, there was no significant difference in BW, ADG, ADFI, and G:F ratio. It has been reported that there is an effect of improving.

When comparing the ZL treatment with zinc oxide and low probiotic addition to the NH treatment with no zinc oxide and high probiotic addition, ADG, ADFI, and G:F ratio of the NH treatment in Phase II numerically was high.

In conclusion, it is thought that the decrease in the level of zinc oxide in the feed for weaning pig and the addition of 0.1% probiotics had a positive effect on the growth performance.

Fecal microflora

Fig. 1, Fig. 2 and Fig. 3 show the effect of the addition of zinc oxide and probiotics in the feed for weaning pig on the fecal microflora. Figure 1 shows quantitative experiments for Aerobic count (AC), Coliform count (CC), *E. coli*/coliform count (EC), Lactic Acid Bacteria count (LAB), and Yeast and Mold count (YM). Figure 2 shows EC, YM, and LAB according to zinc addition. Figure 3 shows the relative abundance of intestinal microbes including Proteobacteria and Lactobacillus.

As a result of quantitative experiment, there was a significant difference in CC and YM. In CC, NH with 0.1% of probiotics without addition of zinc oxide was extremely significantly lower compared to other treatments ($P<0.01$). In the case of YM, ZM added with zinc oxide and 0.05% of probiotics was significantly higher than that of other treatments ($P<0.05$).

As a result of quantitative experiments on the Zinc Oxide-added group in the feed for weaning piglets, there were significant differences in EC, YM and LAB ($P<0.05$). In the case of EC and YM, the treatment with zinc oxide was significantly higher than that of the treatment without zinc oxide. Conversely, in the case of LAB, the treatment group with zinc oxide was significantly lower than the treatment group without the addition of zinc oxide.

As a result of analyzing the effect of the addition of zinc oxide and probiotics in the feed for weaning piglets on the relative abundance, in the case of Lactobacillus, it was found that the tendency of Lactobacillus to decrease in ZL, ZM, and ZH treatments with zinc oxide added. In addition, in the case of NL, NM, and NH treatments without zinc oxide, Lactobacillus in feces increased when the probiotics was fed at 0.01%, 0.05%, and 0.10%. There was no significant difference in Proteobacteria, a phylum containing pathogenic microorganisms such as *E. coli*, Salmonella, and Shigella. However, when 0.1% of the probiotic was added without zinc oxide in the feed for weaning pig, Proteobacteria was found to be the lowest numerically.

According to IHARA et al. (2020), when probiotics were added in the feed for weaning pig, pathogenic bacteria, including *E. coli*, were significantly lower in feces compared to treatments without probiotics. According to Dong et al (2013), when feeding *Lactobacillus plantarum GF103*, *Bacillus subtilis B27*, *Lactobacillus plantarum GF103* and *Bacillus subtilis B27* in weaning piglets, *E. coli* significantly decreased and beneficial bacteria such as *Lactobacillus plantarum* and *Bacillus subtilis* significantly increased.

According to a study by Waern et al. (1998), it was reported that there was no significant difference in *E. coli* in feces when zinc oxide was added to the feed for weaning piglets. This showed the same results as in this experiment.

In conclusion, the addition of 0.1% probiotics in the feed for weaning piglets has a positive effect on the increase of beneficial bacteria in the intestines.

Diarrhea index

Table 4 shows the effect of the addition of zinc oxide and probiotics in the feed for weaning pig on the diarrhea index. As a result of this experiment, the treatment groups ZL, ZM, and ZH containing zinc oxide in the feed for weaning pig significantly decreased at 3 and 6 weeks compared to NL, NM, and NH treatments without the addition of zinc oxide. ($P<0.01$).

However, although there is a difference in the diarrhea index numerically, it is not considered to be a big difference when management in an actual farm.

In general, diarrhea occurs over a long period of time, such as 1-2 weeks, due to changes in feed after weaning and changes in the digestive system. Specifically, this is due to the fact that the height of the villi becomes shorter, the depth of the crypt becomes deeper, and the function of digestive enzymes decreases, resulting in a decrease in nutrient absorption capacity (Spreeuwenberg et al., 2001). In addition, a decrease in absorption capacity in the small intestine is associated with the growth of pathogenic bacteria or a decrease in the fermentation of digestible nutrients in the large intestine (McCracken and Kelly, 1993), which induces diarrhea in weaning pig.

In a previous study by Waern et al. (1998), it was reported that during the first period of weaning pig, the intestinal villi of weaning pigs recovered significantly, and the incidence of diarrhea decreased in the later period of weaning pig. Therefore, in this experiment, as in previous studies, the amount of zinc oxide added in the phase II feed was reduced, but the incidence of diarrhea was thought to have decreased.

In addition, according to Zani et al. (2002), various probiotics such as *Lactobacillus casei*, *Bacillus cereus*, *Bacillus licheniformis*, and *Bacillus subtilis* are generally believed to reduce the incidence of diarrhea in piglets by making beneficial bacteria dominate and stabilize the intestinal microflora in the large intestine. According to Hu et al., (2014), when *Bacillus subtilis* was added at levels of 2×10^9 CFU/kg, 4×10^9 CFU/kg, and 20×10^9 CFU/kg in the weaning pig feed, diarrhea occurred less than that of the treated group without addition, and reported that the level was similar to that of the treated group with antibiotics.

As a result of this experiment, it was the same as the previous study that diarrhea decreased in the treatment with zinc oxide (Hahn and Baker, 1993; Hill et al., 2001). According to a previous study by Burch (2014), diarrhea decreased when 2000 – 4000 ppm was added as a pharmacological level.

In conclusion, it seems that the addition of zinc oxide in the feed for weaning pig has a positive effect on diarrhea reduction, and the addition of probiotics does not have a negative effect.

Immune response

Table 5 shows the effect of the addition of zinc oxide and probiotics in the feed for weaning pig on the immunity of weaning pig. Blood was collected at the beginning and end of each feeding phase and analyzed for immune IgA.

Blood was collected at the end of each feeding step and analyzed for IgA in serum, but there was no significant difference. However, the concentration of IgA in blood was numerically higher in the 3rd and 6th weeks in the treatment groups NL, NM, and NH added with the probiotic compared to the ZL, ZM, and ZH treatment groups with zinc oxide in the feed of weaning pig.

According to Chai et al. (2014), there is a previous study that showed that there was no significant difference in the concentration of IgA when 3,100 mg/kg of zinc oxide was added to the feed for weaning pig. On the contrary, it was reported that IgA increased in the treatment with 3,100 mg/kg of zinc oxide in feed for weaning pig (Broom et al., 2005).

According to Naquid et al., (2014), when the probiotic *Lactobacillus plantarum* was added in the feed for weaning pig, IgA was reported to be significantly increased. In addition, according to Dong et al., (2013), it was reported that the concentration of IgA was significantly increased when the probiotics *Lactobacillus plantarum GF103*, *Bacillus subtilis B27*, *Lactobacillus plantarum GF103* and *Bacillus subtilis B27* were added in the feed for weaning pig.

In conclusion, the addition of probiotics in the weaned pig diet seems to have a positive effect on immunity.

Conclusion

This study was conducted to determine the effect of the addition of zinc oxide and probiotics in the feed for weaning piglets on the growth performance, blood profiles, immune response, diarrhea index and fecal microflora in weaning pigs.

As a result of the experiment, BW of ZL, ZM, and ZH treated with zinc oxide was significantly higher compared to NL, NM, and NH of treatments not ingested at week 3 and week 6 ($P<0.01$). In the case of ADG, ZL, ZM, and ZH treated with zinc oxide were significantly higher in Phase I and the entire period compared to NL, NM, and NH without ingestion ($P<0.01$). In the case of ADFI, ZL, ZM, and ZH treated with zinc oxide were significantly higher in Phase I and the entire period compared to NL, NM, and NH without intake of zinc oxide ($P<0.01$). In the case of G;F ratio, ZL, ZM, and ZH treated with zinc oxide were significantly higher in Phase I and all periods than NL, NM and NH without ingestion ($P<0.01$).

Result of quantitative experiment in branching microorganisms, there was a significant difference in CC and YM., In CC, NH with 0.1% of probiotics without addition of zinc oxide was extremely significantly lower compared to other treatments ($P<0.01$). In the case of YM, ZM added with zinc oxide and 0.05% of probiotics was significantly higher than that of other treatments ($P<0.05$). In the case of Zinc-Quantitative experiment, *E. coli*/coliform count (EC) and yeast & mold (YM) were significantly higher and lactic acid bacteria count (LAB) was significantly lower in the treatment with zinc oxide ($P<0.05$). However, in the case

of NL, NM, and NH treatments without zinc oxide, Lactobacillus in feces increased when probiotics were added at each level of 0.01%, 0.05%, and 0.10%.

The diarrhea index was significantly lower at 3 weeks and 6 weeks than in the treatment groups ZL, ZM, and ZH without the addition of zinc oxide, compared to NL, NM, and NH ($P<0.01$).

There was no significant difference in immunity. However, NL, NM, and NH, which added only probiotics in the weaning pig feed, had higher blood IgA concentrations at week 3 and week 6 than ZL, ZM, and ZH with zinc oxide added to the weaned pig feed.

In conclusion, the addition of zinc oxide in the feed for weaning piglets has a positive effect on growth performance and diarrhea index, and the addition of 0.1% probiotics increases the activity of the beneficial bacteria *Bacillus subtilis*, increases the concentration of IgA in the blood, and is positive in G:F ratio. Therefore, it is expected that probiotics can partially replace zinc oxide.

Table 1. The Formulas and chemical composition of the phase Ⅰ diet

Item	Treatment[1]					
	NL	NM	NH	ZL	ZM	ZH
Ingredients (%)						
Corn	40.14	40.08	39.98	39.69	39.61	39.52
Expanding corn	10.00	10.00	10.00	10.00	10.00	10.00
SBM	34.37	34.37	34.39	34.42	34.44	34.45
Soy oil	0.21	0.23	0.26	0.35	0.38	0.41
Sweet whey powder	4.00	4.00	4.00	4.00	4.00	4.00
Lactose	8.00	8.00	8.00	8.00	8.00	8.00
L-Lysine-HCl, 78%	0.27	0.27	0.27	0.27	0.27	0.27
DL-met, 80%	0.05	0.05	0.05	0.05	0.05	0.05
L-threonine, 99%	0.10	0.10	0.10	0.10	0.10	0.10
MDCP	1.42	1.42	1.42	1.42	1.42	1.42
Limestone	0.93	0.93	0.93	0.93	0.93	0.93
Vit. Mix	0.10	0.10	0.10	0.10	0.10	0.10
Min. Mix	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Probiotics[2]	0.01	0.05	0.10	0.01	0.05	0.10
ZnO	0.00	0.00	0.00	0.25	0.25	0.25
Sum	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)[3]						
ME (kcal/kg)	3265.00	3265.00	3265.00	3265.00	3265.00	3265.00
Crude protein (%)	20.56	20.56	20.56	20.56	20.56	20.56
Lysine (%)	1.35	1.35	1.35	1.35	1.35	1.35
Methionine (%)	0.35	0.35	0.35	0.35	0.35	0.35
Threonine (%)	0.86	0.86	0.86	0.86	0.86	0.86
Calcium (%)	0.80	0.80	0.80	0.80	0.80	0.80
Phosphorus (%)	0.65	0.65	0.65	0.65	0.65	0.65

[1] NL : Basal diet + probiotics 0.01 %, NM : Basal diet + probiotics 0.05 %, NH : Basal diet + probiotics 0.1 %, ZL : Basal diet + probiotics 0.01 % + ZnO 0.25 %, ZM : Basal diet + probiotics 0.05 % + ZnO 0.25 %, ZH : Basal diet + probiotics 0.1 % + ZnO 0.25 %.

[2] *Bacillus coagulance* $1X10^9$ 이상 CFU/g, *Bacillus lichenformis* $5X10^8$ 이상 CFU/g, *Bacillus subtilis* $1X10^9$ 이상 CFU/g, *Clostridium butyricum* $1X10^8$ 이상 CFU/g

[3] Calculated value.

Table 2. The Formulas and chemical composition of the phase Ⅱ diet

Item	Treatment[1]					
	NL	NM	NH	ZL	ZM	ZH
Ingredients (%)						
Corn	56.00	55.93	55.84	55.94	55.88	55.79
Expanding corn	5.00	5.00	5.00	5.00	5.00	5.00
SBM	30.05	30.06	30.07	30.07	30.07	30.08
Soy oil	0.15	0.17	0.20	0.17	0.19	0.22
Sweet whey powder	2.00	2.00	2.00	2.00	2.00	2.00
Lactose	4.00	4.00	4.00	4.00	4.00	4.00
L-Lysine-HCl, 78%	0.17	0.17	0.17	0.17	0.17	0.17
DL-met, 80%	0.02	0.02	0.02	0.02	0.02	0.02
L-threonine, 99%	0.03	0.03	0.03	0.03	0.03	0.03
MDCP	1.26	1.26	1.26	1.26	1.26	1.26
Limestone	0.81	0.81	0.81	0.81	0.81	0.81
Vit. Mix	0.10	0.10	0.10	0.10	0.10	0.10
Min. Mix	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Probiotics[2]	0.01	0.05	0.10	0.01	0.05	0.10
ZnO	0.00	0.00	0.00	0.025	0.025	0.025
Sum	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition (%)[3]						
ME (kcal/kg)	3265.00	3265.00	3265.00	3265.00	3265.00	3265.00
Crude protein (%)	18.88	18.88	18.88	18.88	18.88	18.88
Lysine (%)	1.15	1.15	1.15	1.15	1.15	1.15
Methionine (%)	0.30	0.30	0.30	0.30	0.30	0.30
Threonine (%)	0.74	0.74	0.74	0.74	0.74	0.74
Calcium (%)	0.70	0.70	0.70	0.70	0.70	0.70
Phosphorus (%)	0.60	0.60	0.60	0.60	0.60	0.60

[1] NL : Basal diet + probiotics 0.01 %, NM : Basal diet + probiotics 0.05 %, NH : Basal diet + probiotics 0.1 %, ZL : Basal diet + probiotics 0.01 % + ZnO 0.25 %, ZM : Basal diet + probiotics 0.05 % + ZnO 0.25 %, ZH : Basal diet + probiotics 0.1 % + ZnO 0.25 %.

[2] *Bacillus coagulance* $1X10^9$ 이상 CFU/g, *Bacillus lichenformis* $5X10^8$ 이상 CFU/g, *Bacillus subtilis* $1X10^9$ 이상 CFU/g, *Clostridium butyricum* $1X10^8$ 이상 CFU/g

[3] Calculated value.

Table 3. Effects of Zinc and probiotics in weaning pig diet on growth performance

Criteria	Treatment[1]						SEM[2]	*P*-value		
	NL	NM	NH	ZL	ZM	ZH		Zn	Pro	Zn*Pro
Body weight, kg										
Initial	6.67	6.67	6.67	6.67	6.66	6.67	0.154	0.99	1.00	1.00
3 week	8.39[b]	8.24[b]	8.13[b]	10.04[a]	9.44[a]	9.99[a]	0.236	<0.01	0.75	0.79
6 week	12.39[b]	12.98[b]	12.99[b]	14.45[a]	15.20[a]	14.63[a]	0.336	<0.01	0.67	0.92
Average daily gain, g										
0-3 week	82.07[b]	74.80[b]	69.55[b]	160.69[a]	132.38[a]	158.43[a]	8.113	<0.01	0.23	0.30
3-6 week	190.81	225.93	231.96	210.22	274.35	274.35	9.838	0.33	0.12	0.45
0-6 week	272.88[b]	300.73[b]	301.52[b]	370.91[a]	406.73[a]	379.46[a]	13.529	<0.01	0.50	0.87
Average daily feed intake, g										
0-3 week	213.50[b]	196.90[b]	192.95[b]	265.24[a]	235.55[a]	275.24[a]	7.670	<0.01	0.21	0.26
3-6 week	297.81	325.52	341.71	321.27	391.63	391.63	9.525	0.09	0.08	0.31
0-6 week	511.32[b]	522.42[b]	534.66[b]	586.51[a]	627.18[a]	617.00[a]	12.636	<0.01	0.49	0.83
Gain : Feed ratio (G:F ratio)										
0-3 week	0.388[b]	0.386[b]	0.355[b]	0.609[a]	0.563[a]	0.574[a]	0.0254	<0.01	0.73	0.85
3-6 week	0.638	0.693	0.677	0.653	0.699	0.636	0.0157	0.83	0.43	0.76
0-6 week	0.534[b]	0.572[b]	0.564[b]	0.632 [a]	0.646[a]	0.612[a]	0.0126	<0.01	0.60	0.66

[1] NL : Basal diet + probiotics 0.01 %, NM : Basal diet + probiotics 0.05 %, NH : Basal diet + probiotics 0.1 %, ZL : Basal diet + probiotics 0.01 % + ZnO 0.025 %, ZM : Basal diet + probiotics 0.05 % + ZnO 0.025 %, ZH : Basal diet + probiotics 0.1 % + ZnO 0.025 %

[2] Standard error of means.

Table 4. Effects of Zinc and probiotics in weaning pig diet on diarrhea index

Criteria	Treatment[1]						SEM[2]	*P*-value		
	NL	NM	NH	ZL	ZM	ZH		Zn	Pro	Zn*Pro
Fecal consistency score										
3 week	1.88 ^{abc}	2.32 ^{a}	1.98 ^{ab}	1.74 ^{abc}	1.34 ^{c}	1.38 ^{bc}	0.3407	<0.01	0.77	0.19
6 week	1.24^{a}	1.36^{a}	1.44^{a}	0.50^{b}	0.64^{b}	0.46^{b}	0.4144	<0.01	0.74	0.69

[1] NL : Basal diet + probiotics 0.01 %, NM : Basal diet + probiotics 0.05 %, NH : Basal diet + probiotics 0.1 %, ZL : Basal diet + probiotics 0.01 % + ZnO 0.025 %, ZM : Basal diet + probiotics 0.05 % + ZnO 0.025 %, ZH : Basal diet + probiotics 0.1 % + ZnO 0.025 %

[2] Standard error of means.

Table 5. Effects of Zinc and probiotics in weaning pig diet on immune response

Criteria	Treatment[1]						SEM[2]	*P*-value		
	NL	NM	NH	ZL	ZM	ZH		Zn	Pro	Zn*Pro
Serum IgA (μg/ml)										
Initial	- - - - - - - - - - 346.19 - - - - - - - - -						-	-	-	-
3 week	1158.73	1146.26	1141.60	961.10	749.94	880.76	155.270	0.06	0.80	0.84
6 week	1955.60	1321.59	1848.09	1648.31	1314.27	1636.36	240.915	0.52	0.30	0.90

[1] NL : Basal diet + probiotics 0.01 %, NM : Basal diet + probiotics 0.05 %, NH : Basal diet + probiotics 0.1 %, ZL : Basal diet + probiotics 0.01 % + ZnO 0.025 %, ZM : Basal diet + probiotics 0.05 % + ZnO 0.025 %, ZH : Basal diet + probiotics 0.1 % + ZnO 0.025 %

[2] Standard error of means.

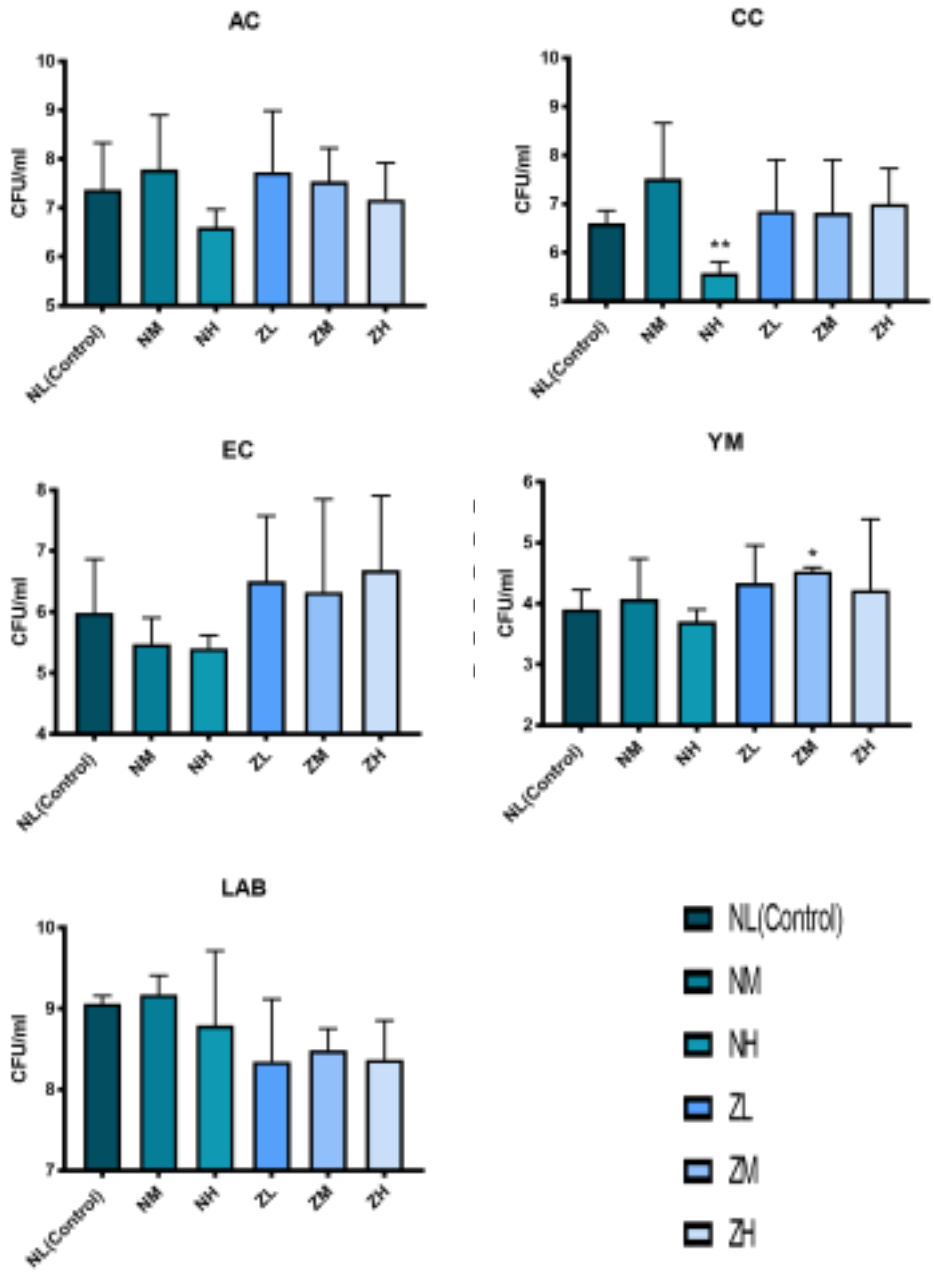

Figure 1. Effects of zinc and probiotics in weaning pig diet on fecal microflora (Quantitative experiment)

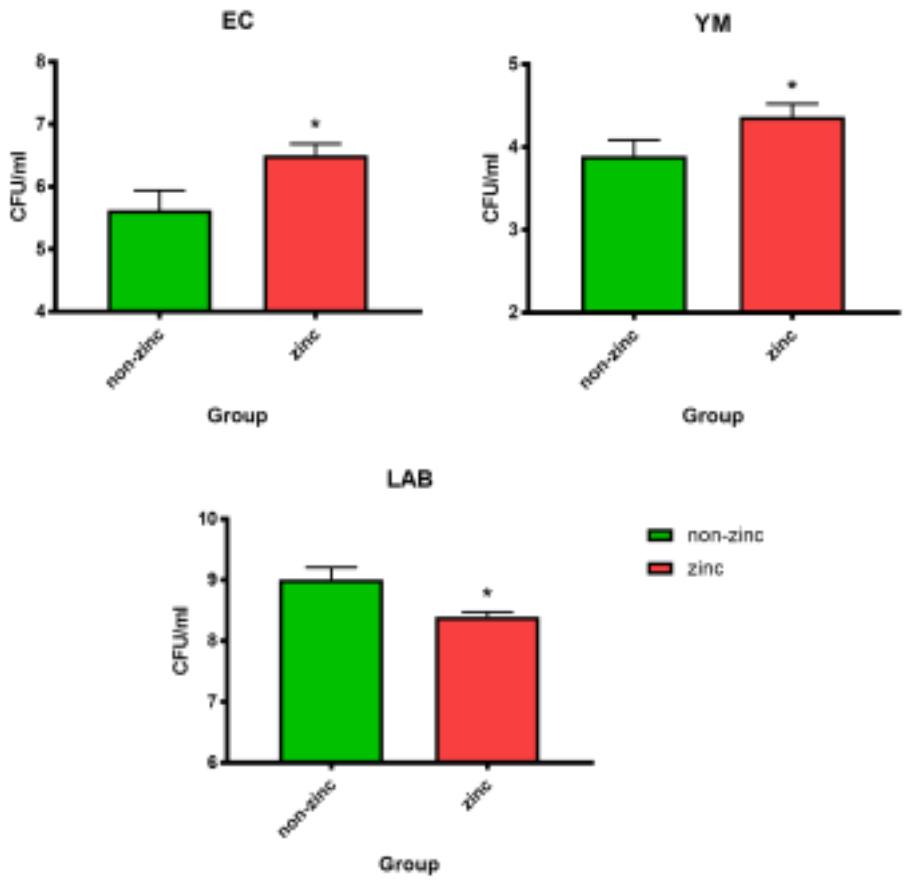

Figure 2. Effects of zinc and probiotics in weaning pig diet on fecal microflora (Zinc-quantitative experiment)

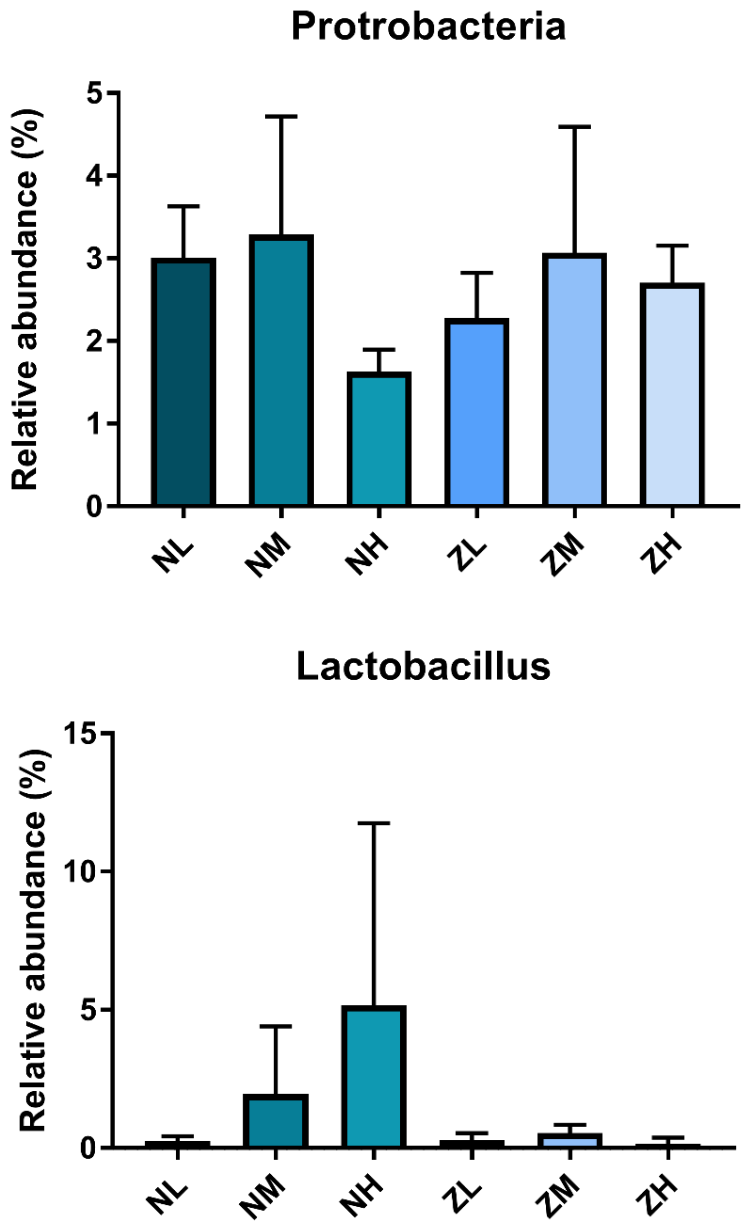

Figure 3. Effects of zinc and probiotics in weaning pig diet on fecal microflora (Relative abundance)

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Ⅴ. Summary in Korean

본 실험은 사료 내 산화아연과 생균제 첨가가 이유자돈에 미치는 영향을 조사하기 위해 수행되었다. 28 ± 2 일령에 이유한 평균체중 6.67 ± 0.872 kg의 삼원교잡종 [(Yorkshire × Landrace) × Duroc] 이유자돈 300두를 공시하였다. 처리구 당 실험 사료는 1) NL : 옥수수-대두박 위주의 기초 사료 + 생균제 0.01 %, 2) NM : 기본사료 + 생균제 0.05 %, 3) NH : 기본사료 + 생균제 0.1 %, 4) ZL : 기본사료 + 생균제 0.01 % + 산화아연, 5) ZM : 기본사료 + 생균제 0.05 % + 산화아연, 5) ZH : 기본사료 + 생균제 0.1 % + 산화아연으로 구성되었으며 이유자돈 전기의 산화아연 첨가량은 0.25 %이고 후기는 0.025 %이다. 전체 6주간의 실험의 결과 성장성적에서 산화아연을 섭취한 이유자돈의 경우 체중에서 3 주차와 6 주차에 섭취하지 않은 경우와 비교했을 때 유의적으로 높았다 (P<0.01). 일당증체량의 경우 산화아연을 첨가한 처리구 ZL, ZM, ZH가 첨가하지 않은 처리구 NL, NM, NH와 비교했을 때 Phase I 과 전체 기간에서 유의적으로 높았다 (P<0.01). 일당사료섭취량의 경우 역시 산화아연을 첨가한 처리구 ZL, ZM, ZH가 첨가하지 않은 처리구 NL, NM, NH와 비교했을 때 Phase I 과 전체 기간에서 구간에서 유의적으로 높았다 (P<0.01). 사료효율의 경우에서도 산화아연을 첨가한 처리구 ZL, ZM, ZH가 첨가하지 않은 처리구 NL, NM, NH와 비교했을 때

Phase I 과 전체 기간에서 유의적으로 높았다 (P<0.01). 분변내 미생물의 경우 산화아연을 첨가한 처리구에서 대장균과 효모 및 곰팡이는 유의적으로 낮았고 유산균은 유의적으로 높았다 (P<0.05). 또한 정량 실험의 결과 대장균군의 경우 NH가 유의적으로 낮았으며 (P<0.01) 효모 및 곰팡이의 경우 ZM이 유의적으로 높았다(P<0.05). 설사지수에서는 산화아연을 첨가한 처리구 ZL, ZM, ZH가 첨가하지 않은 처리구 NL, NM, NH와 비교했을 때, 3주와 6주에서 모두 유의적으로 낮았다 (P<0.01). 면역 성상에서는 IgA의 경우 유의적인 차이를 보이지 않았으나 수치적으로 생균제만 첨가한 처리구에서 산화아연을 첨가한 처리구 보다 IgA가 높았다.

결과적으로, 자돈사료 내 산화아연의 첨가가 성장성적과 설사지수에 긍정적인 영향을 미치나 산화아연을 첨가하지 않고 생균제를 처리한 첨가구가 IgA와 장내 미생물에 긍정적인 영향을 미치기 때문에 보상성장과 추가적인 2차 효과를 기대할 수 있을 것으로 사료된다.