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For the Degree of Master of Science

Effect of Different Type of Nucleotide Supplementation on Growth Performance, Blood Profile, Diarrhea Score and *E.coli* Challenge in Weaning Pigs Diets

자돈 사료 내 종류별 핵산 첨가가 자돈의 성장성적, 혈액성상 및 설사빈도에 미치는 영향과 자돈 사료 내 종류별 핵산 첨가가 공격접종 된 이유자돈의 해부학적 특성 및 설사에 미치는 영향 비교

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지도교수 **김 유 용**

이 논문을 농학석사 학위논문으로 제출함

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김 지 민

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Summary

Weaning is one of the most stressful phase for piglets. Because of weaning stress, weaning pigs are generally showed a reducing digestion, absorption of nutrients and increase immune challenges, oxidative stress, or diarrhea, which in turn intervenes with feed intake and growth performance (Salobir et al., 2005; Kim et al., 2010). It would affect commercial farms productivity by causing disease and an increment of mortality of pigs. Until a decade ago, antibiotic has been widely used as AGP (antibiotic growth promoter) to improve productivity of commercial farms with increasing feed efficiency, promoting growth performance, decreasing incidence of diarrhea and enhancing the quality of animal diets. However, AGPs have been prohibited in 1986 from Sweden for the first time in the world and forbidden in the EU in 2006. In Korea AGPs were also prohibited from July 1st in 2011 because antibiotic resistant-bacteria were found in humans and livestock. The swine industry has been looking for some potential alternatives for in-feed AGPs in starter diets of pig to alleviate weaning-associated growth retardation. Among a number of alternatives such as probiotics, prebiotics, enzymes and zinc, nucleotide has been proposed to overcome occurrences of diarrhea in weanling pigs those are most often related to infections with bacteria such as *E.coli.* However, very lack of information is available related with nucleotide especially purine nucleotide effects on immune system, growth performance and intestinal health for young animals. Therefore an experiment was conducted to evaluate an effect of different

type of nucleotide supplementation on growth performance, blood profile and diarrhea score in weaning pigs (Exp. 1) and effect of IMP (Inosine Mono-Phosphate) and GMP (Guanosine Mono-Phosphate) use on anatomic trait and diarrhea of weaning pigs under *E.coli* challenge was also evaluated (Exp. 2). The Exp. 1 was designed with a total of 288 crossbred ([Yorkshire x Landrace]) x Duroc) pigs (7.17 ± 1.176kg BW) were allotted into six treatments considering sex and initial body weight in 6 replication with 8 pigs per pen in a randomized complete block (RCB) design. Treatments were as followed CON: corn-soybean meal basial diet + ZnO 500 (Phase I) / 300ppm (Phase II); I5: corn-soybean meal basial diet + IMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); I10: corn-soybean meal basial diet + IMP 0.10% + ZnO 500 (Phase I) / 300ppm (Phase II); IG2.5: corn-soybean meal basial diet + IMP 0.025% + GMP 0.025% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5: corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5a: corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm (Phase I / Phase II). The supplementing nucleotide showed positive results in growth performance especially IMP 0.025% + GMP 0.025% and IMP 0.05% revealed the best growth performance among treatments. In blood profiles of EXP 1, there was no significant difference in immunoglobulins (IgG, IgA and IgM), TNF-α and IL-6 among treatments. In conclusion, supplementing IMP 0.025% + GMP 0.025% showed the highest results in BW, ADG, ADFI, and G:F ratio. The Exp. 2 was designed with a total of 45 crossbred ([Yorkshire x Landrace]) x Duroc) pigs (8.84 ± 0.20kg BW) were allotted into one of five treatments considering sex and initial body weight with 9

pigs per pen in randomized complete block (RCB) design. Treatments were as followed I5: Corn-soybean meal basial diet + IMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); I10: corn-soybean meal basial diet + IMP 0.10% + ZnO 500 (Phase I) / 300ppm (Phase II); IG2.5: corn-soybean meal basial diet + IMP 0.025% + GMP 0.025% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5: corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5a: corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm (Phase I / Phase II). Weaning pigs were orally challenged with *E.coli* K88. Supplementing IMP 0.025% + GMP 0.025% improved immune system and intestinal morphology in weanling pigs. Nutrient digestibility had no significant difference among treatments. The piglets were fed IMP 0.025% + GMP 0.025% mostly had greater villus height and crypt depth compared with control treatment group. No significant difference was observed in the incidence of diarrhea among treatments. Consequently, morphological changes in small intestine, such as longer villus height and deeper crypt depth, were observed compared to other treatments in general was highly affected by supplementation of IMP 0.025% + GMP 0.025% to show the most positive effects on nutrient absorption and utilization.

Keywords : Nucleotide, IMP(Inosine Mono-Phosphate), GMP(Guanosine Mono-Phosphate), Weaning pig, Growth performance, Intestinal morphology, *E.coli* K88

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Ⅰ. Review of Literature

List of Abbreviation

AA	Amino acids
ADFI	Average daily feed intake
ADG	Average daily gain
ADP	Adenosine Mi-Phosphate,
AGP	Antibiotic Growth Promoter
AMP	Adenosine Mono-Phosphate
AOAC	Association of official analytical chemists
ATP	Adenosine Tri-Phosphate,
BW	Body weight
CP	Crude protein
CRD	Completely randomized design
CTP	Cytidine Tri-Phosphate.
DM	Dry matter
DNA	Deoxyribonucleic acid
E.coli	Escherichia coli
EU	European union
G:F	Gain : feed
GDP	Guanosine Di-Phosphate,
GMP	Guanosine Mono-Phosphate,
GTP	Guanosine Tri-Phosphate,
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin-6
GMP	Guanosine Mono-Phosphate
IMP	Inosine Mono-Phosphate
ME	Metabolizable energy
MUFA	Mono unsaturated fatty acid
NRC	National research council

OMP	Orotidine Mono-Phosphate,
PUFA	Polyunsaturated fatty acid
RCB	Randomized complete block
RNA	Ribonucleic acid
SAS	Statistical analysis system
SBM	Soybean meal
SFA	Saturated fatty acid
SO	Soybean oil
TER	Transepithelial electrical resistance
TNF-α	Tumor necrosis factor - alpha
UDP	Uridine Di-Phosphate,
UMP	Uridine Mono-Phosphate,
UTP	Uridine Tri-Phosphate,

I. Introduction

Weaning is one of the most important phase for effective production in commercial farms. However, by making early weaning, piglets easily got stress which is taking place diarrhea, decreasing feed intake and reduction in the length of intestinal villi. In order to overcome weaning-associated problems, farms have been using antibiotics which is having the risk of antibiotic resistance in humans. Due to the potential risk, many countries have already prohibited the use of antibiotics as growth promoter in livestock feeds from 2006 even it has already been widely used in pig production system (D. Martinez-Puig et al., 2007). Additionally, from 2011, Korea has decided to forbid antibiotics in livestock feeds. There are also many different reasons to minimize or completely get rid of the inclusion of in-feed antibiotics in livestock diets (Pluske, 2013). Therefore, finding alternatives to antibiotics is important. Many kinds of substitutes for antibiotics, such as probiotics, prebiotics, oligosaccharides, enzymatic preparations, plant extract, and Chinese medicinal herbs, zinc oxide, nucleotide have been assessed (Li et al., 2015). Among various alternatives, nucleotide are considered as the alternatives because it has positive effects on intestinal morphology, immunity system, smallintestinal growth, and growth performance, high nutrient digestibility and antibacterial effect in pigs. Since weaning is accompanied with morphological, histological, and microbial changes in the gastrointestinal tract of young mammals and yet the amount of *de novo* synthesis of nucleotides is insufficient to meet gut requirements (A Gil et al., 2002).

Consequently, this study was conducted to evaluate the effects of nucleotides supplementation on growth performance, blood profile, diarrhea score in weaning pigs and IMP and GMP use on anatomic trait and diarrhea of weaning pigs under *E.coli* Challenge in weaning pigs.

II. Review of Literature

1. Background

1.1 Problems of post-weaning stress in piglets

Weaning is one of the most stressful phase for piglets. During the weaning, social and dietary stressors can reduce digestion and absorption of nutrients and increase challenges to immune system, oxidative stress, or diarrhea, which in turn reduces feed intake and growth performance (Weaver and Kim, 2014). For instance, moving to a changed environment (pen, building, farm, etc.), separation from the sow and mixing of unfamiliar letters, eating different texture of diets from colostrum to solid feed, experiencing social hierarchy, and increasing exposure to pathogens or environmental contamination are general examples (Richard B. D'Eath., 2004). As a consequence, weaning imposes tremendous stress on piglets and is accompanied by marked changes in gastrointestinal physiology, microbiology and immune system disorders which result in reduced pig health, growth, and feed intake, particularly during the first week after weaning (Campbell et al., 2013).

Particularly, weaning stress can affect growth performance of pigs by reducing feed intake and feed efficiency that connected directly to farms productivity. Currently, in pig production system, weaning is an isolated event that takes place for less than two weeks and it is usually done by abruptly separating the sow from their piglets around the third and fourth week of age (Número especial Vet. Méx., 2014). While weaning, piglets consume sow's milk. However, as soon as piglets finish to feed highly

palatable liquid milk from its sow, the piglet should get used to new solid dry diets abruptly that is less digestible and make damage on intestinal villus (Bruininx et al., 2001). After weaning, villus height is generally decreased and crypt depth increased (Pluske et al., 1996; Cera et al., 1988; Miller et al., 1986), which can cause the increased incidence of diarrhea (Weaver and Kim, 2014). Once gut morphology is changed, it principally related with the low feed intake and growth performance immediately after weaning (Kelly et al., 1991; Pluske et al., 1996)

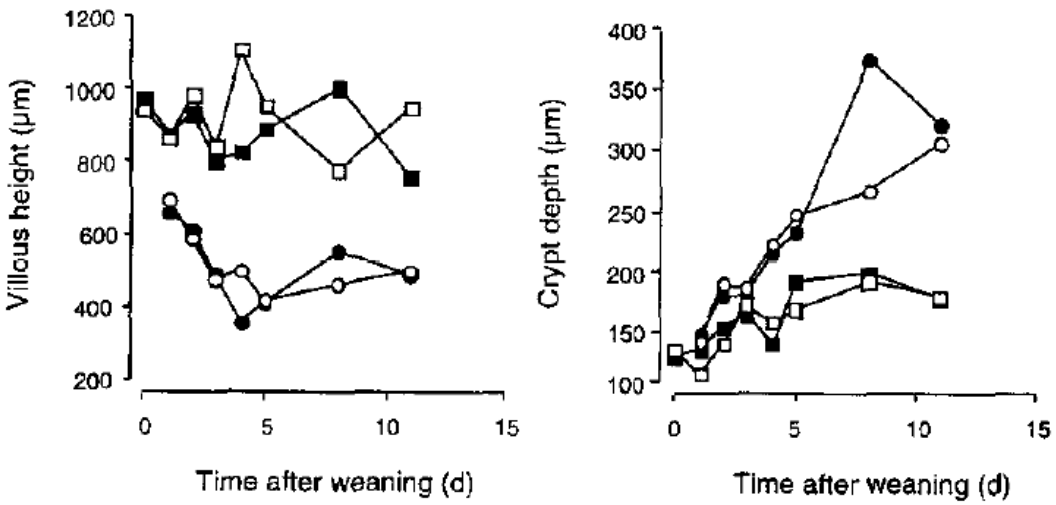

Fig. 1. Villous height and crypt depth at a site 25% along the length of the small intestine of weaned and unweaned pigs killed between 21 and 32 days of age: ○-○ pigs weaned at 21 days of age and offered creep food prior to weaning; ●-● pigs weaned at 21 days of age but not offered creep food prior to weaning; □-□ pigs unweaned and offered creep food; ■-■ pigs unweaned but not offered creep food. Values are means for between two and seven pigs killed per treatment combination per day (redrawn from Hampson, 1986).

Additionally, low feed intake caused changed intestinal morphology is associated with low growth performance. Reflecting decreased brush-border absorption ability and enzyme activity, a decline of intestinal functions are led to the intestinal morphological changes (Vente Spreeuwenberg et al., 2003), which then results in diarrhea and low growth performance in weaning pigs. Also, regardless of weaning age, after weaning, pigs lose about 20-30% of their relative body weight (BW) on the first day, and recovering loss of body weight is taken for at least four days(Le Dividich and Sève., 2000). Since shortening the total days to market (approximately 110 kg of BW) is significant for the farms, the first week after weaning is focused on how much BW the pigs got (Tokach et al.. 1992). This is the reason why paying attention to the feed intake and growth performance of piglets after weaning is crucial.

1.2 Structure and function of the small intestine

In weaning pigs, by switching feed from liquid sow milk to solid feed, low feed consumption, growth check and diarrhea because of the limited capacity of the gastrointestinal tract to utilize and effectively digest starter diets happened in the post-weaning period (Hampson, 1986; Kelly et al., 1990; Efird et al., 1982a,b). In this specific term, a variety of factors may be included in the malfunction of absorption and digestion in weaned piglets related to morphologic and physiological changes in the intestine structure and function of enzymatic activity and absorption or secretion (Miller et al., 1986; Cera et al., 1988; Dusford et al., 1989). These significant changes are highly associated with villus height and crypt depth that affect digestibility and absorption ability of the small

intestine (Pluske, 1997). These changes have been researched by many nutritionists that after weaning, reduction in villous height (villous atrophy) and an increase in crypt depth (crypt hyperplasia) are occurred simultaneously (Hampson, 1986; Pluske et al., 1996; Spreeuwenberg et al., 2001; Boudry et al., 2004; Lalles et al., 2004). The intestinal villi are lengthy anatomically before weaning, efficient for absorbing nutrients and structured well because during lactation, villi are damaged at the least and crypt cells can be replaced villi cells simultaneously when old cells are removed (Nessmith et al., 1997). However, after weaning, height is declined more than a half and crypt depth increased that causing reduced the nutrient absorption to the intestinal cells (D.M. Weary et al., 2007). From this perspective of view, loss of mature enterocytes where digestive enzymes, cell loss are influenced by shortening of the villi (M. S. Hedemann, S. Højsgaard and B. B. Jensen., 2003).

Table 1. Villous height and crypt depth at various sites along the small intestine in piglets killed between 3 days prior to weaning and 9 days post-weaning

	Small intestinal length (%)	Day after weaning				
		-3	0	1	2	3
Villus Height(㎛)	10	413^{ab}	452^{a}	374^{ab}	345^{bc}	292^{c}
	50	369^{ab}	412^{a}	359^{ab}	317^{bc}	264^{c}
	90	300^{ab}	274^{b}	282^{b}	276^{b}	248^{b}
Crypt Depth(㎛)	10	232^{bc}	224^{bc}	202^{c}	223^{bc}	239^{b}
	50	235	212	204	219	235
	90	183^{bc}	162^{c}	176^{bc}	191^{bc}	210^{ab}

Values represent least-squares means and SE of the least-squares means

Values in the same row with different superscripts differ significantly ($p < 0.05$)

1.3 Alternative additives for AGP(Antibiotic Growth Promoters)

Animals growing in commercial farms have become more vulnerable to potentially harmful microorganisms such as *Campylobacter sputorium,* rotavirus, *Clostridium perfringens, Salmonella spp.,* and *Escherichia coli* (Verstegen and Williams., 2002; Thomsson et al., 2008). In order to prevent reducing productivity which cause serious economic losses, the animal feed industry worldwide has been using antibiotics for more than 50 years (Kocher., 2004; H. Vondruskova., 2010). However, people have perceived that the increased use of antibiotics in the swine diets would be harmful due to resistant pathogenic bacterial strains (Budino et al., 2005) and residual contamination of the food chain with antibiotics (Roselli et al., 2005; Van der Fels-Klerx et al., 2011). So that, since 2006, the use of AGP for prevention of a reduction in growth performance and intestinal diseases in piglets has been banned from EU (Li et al., 2015). By doing so, this movement has led to the investigation of alternative suitable feed additives that is effective in protecting and maintaining growth performance and animal health. Various natural substitutes have been researched as efficient AGP such as probiotics (Jacela et al., 2010a; Simon, 2010; Cho et al., 2011), prebiotics (Halas and Nochta, 2012), zinc oxide (Pettigrew, 2006; Jacela et al, 2010b), MCTs (Zentek et al., 2014; Yen et al., 2015) and nucleotide (C.D Mateo and H.H. Stein, 2004; P. Superchi, 2011; D. Martinez-puig et al., 2007).

2. Function of Nucleotide

2.1 Structure of Nucleotide

The first step to understand a structure of Nucleotide, it is important to know the morphological differences among nucleosides, Nucleotides and nucleic acids. A nucleoside is the basic unit of Nucleotide, its structure is various bases bonded (glycosidic bond) to pentose. Bases such as hypoxanthine, adenine, guanine, cytosine, uracil, and thymine, are bonded to pentose and it becomes a nucleosides; inosine, adenosine, guanosine, cytidine, uridine, and thymidine, respectively. Each substance will be classified based on the types of molecule bonding with the second carbon of the pentose.

Ribose (RNA constituent) : hydroxyl group (OH) bonded to the second carbon of pentose

Deoxyribose (DNA constituent) : hydrogen (H) bonded to the second carbon of pentose.

A Nucleotide is formed by phosphate-bonding to a nucleoside. Nucleotides are named according to the number of phosphates (Greek number; mono-, di- or tri-). Adenosine triphosphate (ATP), the biochemical energy for all cells, is a Nucleotide that contains adenosine (base), pentose, and three phosphate groups (Fig. 2).

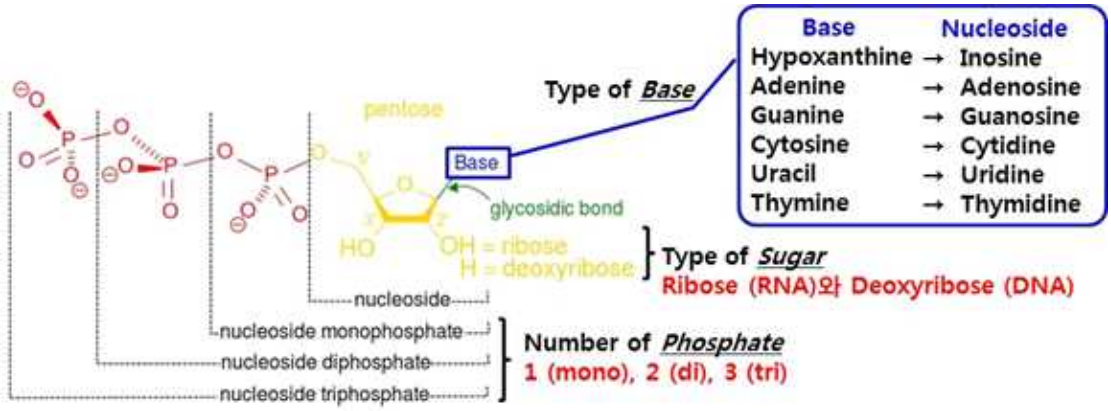

Fig. 2. Types of bases and structures of nucleoside and Nucleotide.

Nucleic acids are composed of Nucleotides that form DNA and RNA depending on types of pentose (deoxyribose or ribose) and a base (Hypoxanthine, Adenine, Guanine, Cytosine, Uracil and Thymine). DNA comprise the chromosomes in cells containing the genomic material. While RNA synthesize diverse proteins require by the cell with amino acids in ribosome (Fig. 3).

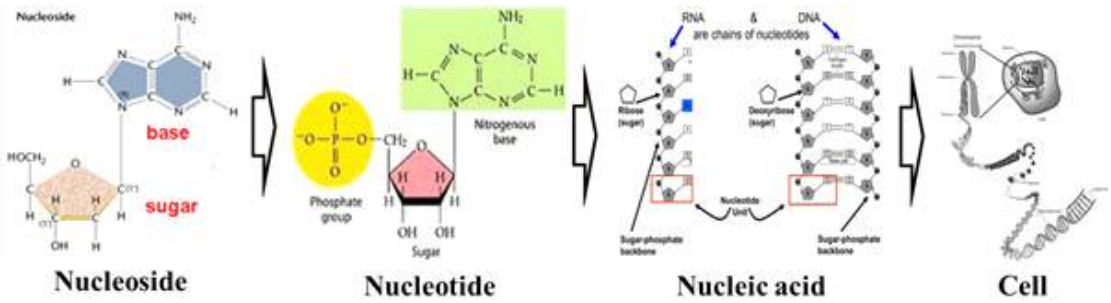

Fig. 3. Process of structural change into intracellular chromosome

2.2 Types of Nucleotide

In order to understand the types of Nucleotide, we need to know about the types and structure of a its base fundamental the primary substance of all nucleotides. Nucleotides are classified into two types

purine or pyrimidine, their classifications can be clarified by molecular structure of the bases as shown in (Fig. 4).

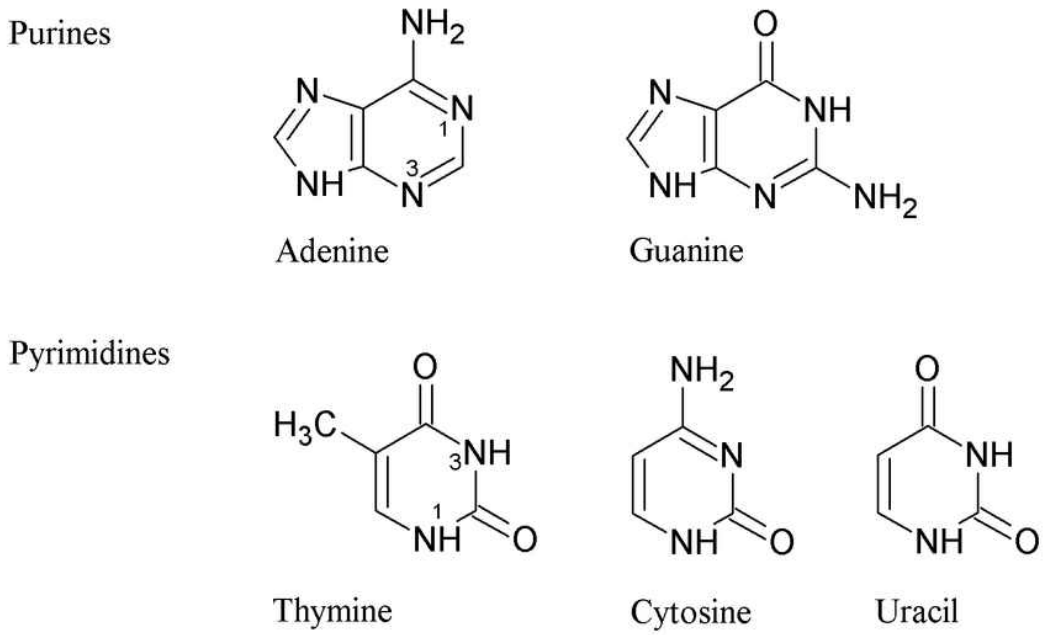

Fig. 4. Molecular structure of purine and pyrimidine Nucleotide

As shown in Fig. 4, the largest molecular structure difference between a purine Nucleotide and a pyrimidine Nucleotide is the binding of the imidazole ring bonded to the heterocycle. It can be classified as a purine if heterocycle is bonded to pentose by imidazole ring and a pyrimidine if heterocycle is directly bonded to pentose without imidazole ring. Therefore, purines contains relatively more carbon and nitrogen atoms for physiological metabolisms than pyrimidines. This implies that carbon and nitrogen are needed raw materials for their synthesis. Especially, in case of nitrogen atoms in bases, they exist as non-nitrogen proteins, which are not considered true proteins. Pyrimidines are receiving their nitrogen for synthesis is from aspartic acids. Purine are receive nitrogen from not only aspartic acid, but also from glutamate and glycine. Although aspartic acid

and glutamate are classified as nonessential amino acids in all livestock, these amino acids play an important roles in energy production and as neurotransmitters. Glycine is classified as an essential amino acid in poultry, and plays an important nutritional and physiological role, such as converting methionine to cysteine as well as functioning as a precursor of threonine. Recently, the nutritional importance of glycine is drawing an attention along with a reduced crude protein (CP) diets when balancing on amino acids. In fact, if CP content of poultry feed is lowered to 20% or less, glycine supplementation should be considered.

If Nucleotides are not supplied sufficiently by feed, both physiological functionality of Nucleotides and nutritional efficiency of the amino acids required for Nucleotide synthesis will decrease. It must be clearly understood about the importance of Nucleotides in metabolic pathways and the excess and deficiency of particular Nucleotides. Unlike essential amino acids which are required to formulate a feed economically, not all Nucleotides are required.

2.3 Metabolic Pathway and Biosynthesis of Nucleotide

As mentioned previously, nucleotides are a non-essential nutrient that can be synthesized in by the body. However, nucleotides are required during periods of rapid growth and organ development (Weaver and Kim, 2014) Additionally for homeostasis increases in case of early growth stages or under-stress conditions such as environmental changes, diseases, injury, handling, or feed with a low nucleotide content (Li et al,. 2015). Since nitrogen and carbon are needed to synthesize nucleotides for growth and maintenance, these elements are required for a protein accumulation.

This could also lead deficiency of fatty acid synthesis (conversion), which may be caused by an insufficient supply of Nucleotides. However, if the Nucleotides are adequately supplied, not only will the Nucleotides needed for metabolism maintenance and growth will be met but also the requirement for the metabolisms of essential nutrients needed for Nucleotide biosynthesis (*de novo* synthesis) becomes unnecessary. Both *salvage pathways* (feed and d*e novo* synthesis) influence Nucleotide biosynthesis. Supply of Nucleotide through *salvage pathway* controls *de novo* synthesis which is controlled by Nucleotide content in the body. Especially, for purine Nucleotides, in which about 90% of the requirement dependent on the *salvage pathway*.

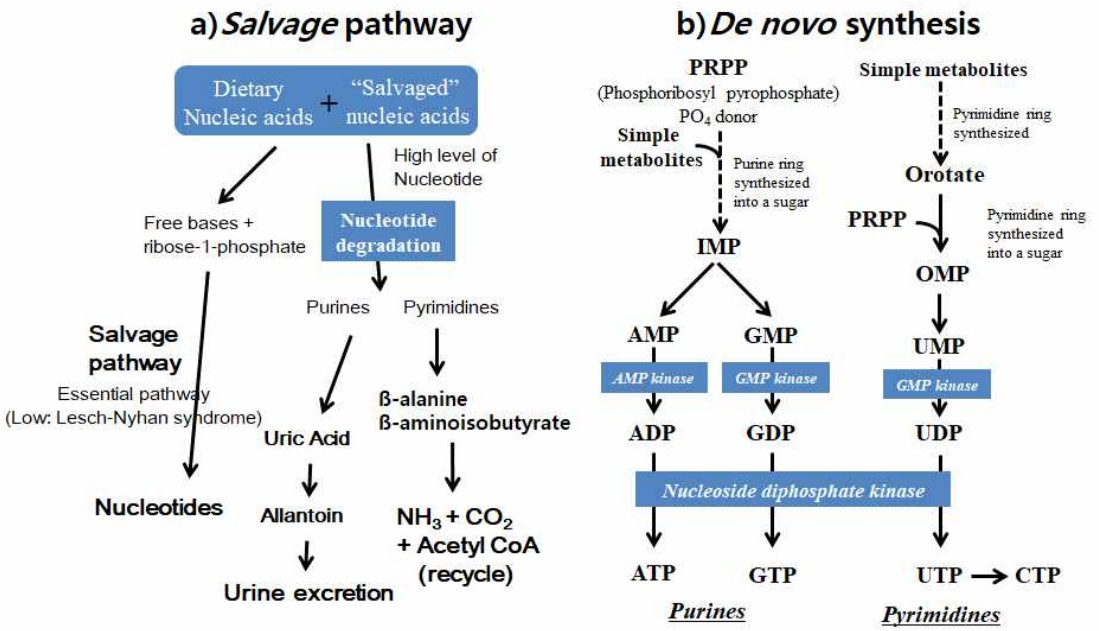

Fig. 5. Biosynthesis pathway of Nucleotide. a) salvage pathway and catabolism of excessive Nucleotide, b) *de novo* synthesis pathway. IMP: Inosine Mono-Phosphate, AMP: Adenosine Mono-Phosphate, ADP: Adenosine Mi-Phosphate, ATP: Adenosine Tri-Phosphate, GMP: Guanosine Mono-Phosphate, GDP: Guanosine Di-Phosphate, GTP: Guanosine Tri-Phosphate, OMP: Orotidine Mono-Phosphate, UMP: Uridine Mono-Phosphate, UDP: Uridine Di-Phosphate, UTP: Uridine Tri-Phosphate, CTP: Cytidine Tri-Phosphate.

As shown in Fig. 5 a), the nucleoproteins or nucleic acids in the feed are digested and absorbed as Nucleotide / nucleoside / base by various digestive enzymes and form a base pool in the body. When the Nucleotides are over-consumed, they are excreted or recycled through different metabolic pathways. In case of purines, the base is converted to uric acid, degraded to allantoin by urate oxidase in liver, and re-synthesize as glyoxylic acid and urea by several enzymes. The

synthesized urea is excreted through the urine, and glyoxylic acid is used in the synthesis of glycine (essential aminoisobutyrate after conversion to uracill and thymine. Finally, NH3, CO2 and Acetyl CoA are synthesized used for pyrimidine resynthesis. Synthesized urea is excreted by urine and glyoxylic acid is utilized in cytosol and mitochondria to synthesize Glycine, an essential amino acid in poultry. On the other hand, pyrimidine types synthesize β-alanine and β-aminoisobutyrate after converted into free bases (uracil and thymine) and eventually used to re-synthesize pyrimidines. However, if the Nucleotide supply via salvage synthesis does not meet the organism's requirement, Nucleotides must be supplied by *de novo* synthesis route as shown in Fig. 5 b). These Nucleotide synthesis pathways are different between purine and pyrimidine. Purine goes through 11 steps requiring 6 ATPs to synthesize IMP (inosine-5'-monophosphate) from ribose-5-phosphate. IMP is then converted to ATP (AMP+phosphate → ADP → ATP) and GTP (GMP+phosphate → GDP → GTP). In addition, another ATP is required to convert IMP to GMP, requiring a total 6 or 7 ATPs to synthesize IMP and GMP via *de novo* synthesis. The *de novo* synthetic pathway of pyrimidines is relatively simple. It requires only 4 steps to produce OMP (Orotidine Monophosphate), a fundamental the base of pyrimidine Nucleotides. It starts by forming carbomoyl phosphate with NH3 and CO2 from glutamine by an enzymatic reaction and requires four less ATPs then are needed in purine pathway. The synthesized OMP is then converted to UMP by an enzymatic reaction and then converted to UTP (UMP → UDP → UTP) by a phosphorylation reaction. Eventually, UTP is converted to CTP by CTP synthetase. Purine and pyrimidine are

essential for cell metabolism Nucleotide that are supplied in the feed, the purines (IMP and GMP) are more important than pyrimidines due to the energy (i.e. ATP) consumption and time required for their biosynthesis.

2.4 Functions of Nucleotide as a Feed Additive

There are many reports on the effect of Nucleotides on the physiological functions (Table 2).

Table 2. Physiological functions of Nucleotides

Energy source	Directly involved in ATP biosynthesis
Coenzyme production	Production of derivatives related to oxidation-reduction of carbohydrate, protein and fat
Physiological regulator	Deliver information to cells (External→Internal)
Carrier synthesis	Transport intermediates involved in glycogen and glycoprotein synthesis and a phospholipid metabolism
Protein synthesis	Synthesize RNA involved in protein synthesis
Mitosis	Increase enzyme activity required for DNA replication in cell division
Lipid metabolism	Promoting an increase of carbon in fatty acids (promoting a conversion of polyunsaturated fatty acid)
Hematopoiesis	Increase oxygen affinity of hemoglobin and stimulation of hematopoiesis
Digestive organ development	Enhancing growth, maturation and recover ability of intestinal epithelial cells
Immunity improvement	Increasing antibody productionsfromT-cell

Also, several studies have reported the nutritional and physiological functions of each Nucleotide and their importance in metabolism and honeostates (Table 3). In this chapter, we will mainly focus on the functions of both purine (adenine hypoxanthine and guanine) and pyrimidine (cytosine, thymine and uracil) bases that comprise DNA and RNA feed additives and their influence on all life-support activities in growth and reproduction of chromosomes in cells.

Table 3. Physiological functions of each Nucleotide

Type		Physiological function
Purine	IMP	Biosynthetic intermediate of all purine *Nucleotide*s (Carver and Walker, 1995)
	GMP	Protein synthesis, microtubule generation and hormone delivery (Pogson, 1974; Huu et al., 2013)
	AMP	Myosin formation, energy supply and Enzyme cofactor of carbohydrate metabolism
Pyrimidine	UMP	Glycogen carrier, Glycoprotein biosynthesis (Huu et al., 2013)
	CMP	Cofactor of enzymes, Phosphoric acid carrier for ADP→ATP translocation
	TMP	UMP Methylation, DNA synthesis inhibition during cell division

2.4.1 Growth Performance / Palatability Enhancement / Digestive Enzyme Secretion Promotion

Functionalities of nucleotide as a feed additives are already verified in different species of animals. Firstly, the Nucleotide (IMP) supplementation in weaning pigs improved feed intake ($p<0.001$) and weight gain ($p=0.031$) significantly when compared to a control-fed group (Weaver and Kim, 2014) at an optimum dosage of 0.05%/diet (Fig. 6). Enhancement immune (IgA, IgM and TNFα) was observed following IMP

supplementation in feed. They also reported that a low dietary intake level of nucleotides in during weaning phase leads to deterioration of growth performance. Furthermore, they also observed a reduced *de novo* IMP synthesis total nucleotide with feed supplemented with IMP.

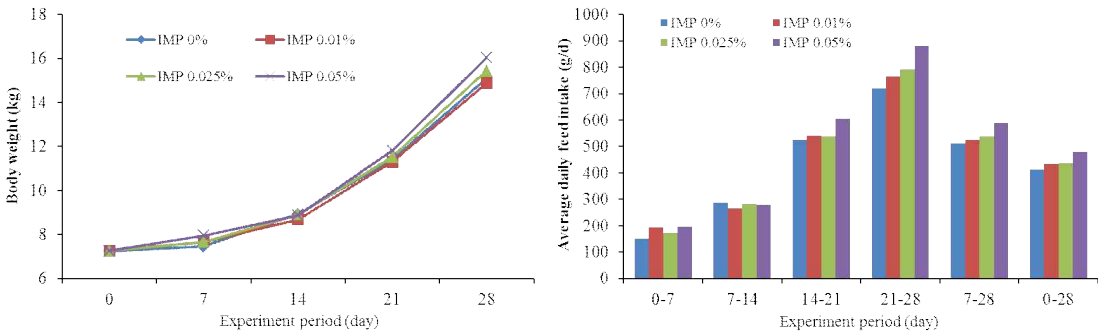

Fig. 6. Effect of IMP supplementation on body weight and average daily feed intake in weaning pigs (22 days, 7.27 kg)

On the other hand, the efficiency of nucleotide in poultry feed are diverse. One thing clear is impact under stress condition such as heat low sanitary or high stocking density (Jung, 2011; Jung and Batal, 2012). The reason is that nucleotide are stressor-specific in case of poultry which do not have the enzymes required for urea cycle or purine Nucleotide metabolism. They utilize ammonia to synthesize amino acids, amines and Nucleotide bases since they cannot produce urea from ammonia. Therefore, like in mammals, the influence of nucleotide ingested exogenously on the energy consumption of nucleotide biosynthesis is relatively small. Nucleotide supply is not considered essential under general conditions (requiring high level of nucleotide). Nevertheless, several researchers are reporting on the effects of Nucleotide supplementation in broiler. For example, the feeding 5% of exogenous

nucleotide in broiler breeders improved egg production rate (1.6% ↑), egg fertility rate (1.7% ↑), embryo hatching rate (4.1% ↑) and fertilized egg hatching rate (2.3% ↑). It also improved meat quality characteristics including a high color intensity and a lowered shear force. Additionally, physical and nutritional characteristics of carcass breast meat, significantly increasing crude lipid contents of monounsaturated fatty acids and linolenic acids (Chiofalo et al., 2011; Table 4).

Table 4. Differences in carcass and meat qualities by Nucleotide supply

	Control	Nucleotides	P-value
physical characteristics			
Redness (a*)	10.82	11.58	< 0.001
Hue (H)	0.86	0.77	< 0.001
Shear value of ground meat (kg/cm^2)	1.18	0.97	< 0.001
Muscle nutritional characteristics (%)			
Ether extract	1.66	1.96	< 0.001
Ash	1.01	1.26	< 0.001
Meat Fatty acids contents (%)			
Σ SFA	40.75	38.55	< 0.001
Σ MUFA	37.29	40.07	< 0.001
Σ PUFA n-3	4.4	3.27	< 0.001

SFA; saturated fatty acid, MUFA; mono unsaturated fatty acid, PUFA; polyunsaturated fatty acid.

Researchers on the effects of nucleotide in aquatic animals are diverse. Aquatic animals are manifested as highly sensitive to nucleotide, especially when they ingested a feeds with vegetable proteins replacing fish meal. Anadromous and strong ichthyophagous fishes are reported to have a high palatability for IMP, the largest part of a fish meal. Kubitza et al., (1997) evaluated the palatability of low fish meal/high SBM diet in largemouth bass (Micropterus salmoides). Among the various attractants

commonly known in aquatic animals, IMP was the most influential on feed intake improvement. Also, using IMP only was more influential than mixing with other palatability enhancing substances (Table 5).

IMP and GMP are widely used as palatability enhancers in food since they are well known as umami enhancing substances which stimulate taste receptors of humans. Previously trial results in aquatic animals imply that IMP may have an identical functions in livestock (at least aquatic animals) like in humans. Also, shrimps as the second largest commercially produced in aquaculture animals, demonstrated a significant growth performance improvement when supplemented Nucleotides in the feed. It was observed that adding just purines was sufficient since purines require lower energy for biosynthesis than pyrimidine which saves energy and amino acids for other metabolic purposes.

Table 5. Changes in palatability of largemouth bass depending on feed additives (Kubitza et al., 1997)

	Daily feed intake (g/100 g body Weight)	Relative DFI* (%)
IMP	**3.6[a]**	**145.8**
IMP + Amino acid + Betaine	**3.45[a]**	**136.7**
IMP + Betaine	**3.42[a]**	**138.5**
IMP + Amino acid	**3.34[a]**	**135.2**
Amino acid + Betaine	2.74[b]	110.9
Amino acid	2.69[b]	108.9
Control (60% Soybean meal diet without fish meal)	2.47[b]	100
Betaine	2.4[b]	97.2

*DFI: daily feed intake

2.4.2 Immunity, Stress Tolerance Effect

Enhancement immune function has been reported as the most prominent among physiological functions of nucleotide. Nucleotides in the cells are involved in immunity such as leukocytes, red blood cells, bone marrow cells, intestinal mucosa cells and lymphocytes are all supplied by the salvage pathway as those synthesized via *de novo* synthesis cannot fulfill the requirement.

In other words, it is necessary to supply adequate nucleotide in the feed to maintain or and enhance immunity.

The immunological effects of Nucleotide are:

(1) Increasing the phagocytosis of non-granular leukocytes such as T-cells and B-cells among various cells involved in immunity

(2) Speeding up the production of antibodies (IgM, IgA) and bone marrow cells

(3) Promoting the production of immune proteins cytokine (IL-2, IFN-g)

(4) Increasing the activation and self-toxicity of NK-cells

Actually, the immunity improvement is observed is an increase in the survival rate during the rearing process and stress tolerance improvement related to diseases and environment changes.

Song et al. (2012) observed changes in survival rate of the flounder (Paralichthys olivaceus) caused by pathogenic attack between the IMP-supplemented and not-supplemented groups. As a result, the survival rate was significantly decreased for the control-fed group on the third day of pathogen aggression. While more than an 80% survival rate was maintained in the IMP group (Fig. 7).

In recent experiments, the carp and salmon, the largest aquaculture

species in freshwater and seawater, were tested for an environmental (salinity change) and handling stress respectively. The survival rate and immunity were improved significantly in IMP-supplemented group in these stress conditions (in press).

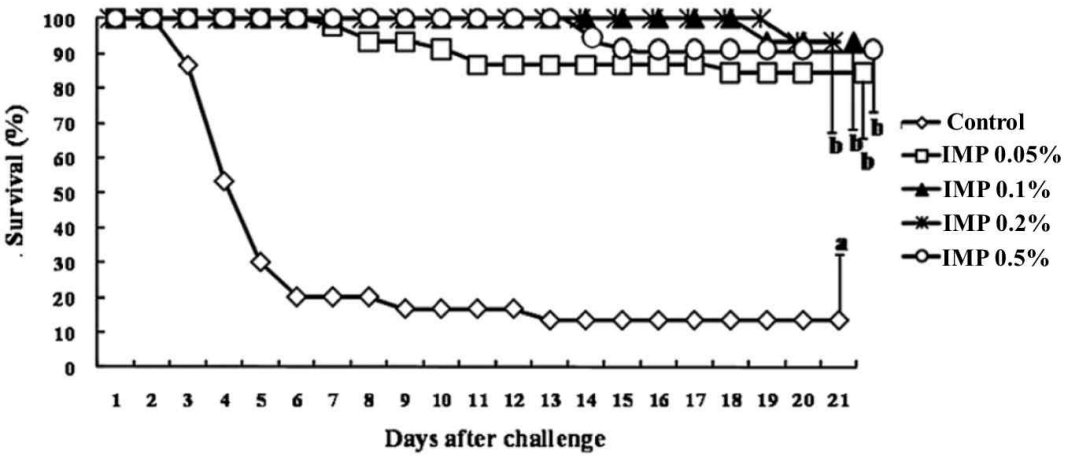

Fig. 7. Cumulative mortality of olive flounder fed five experimental diets containing different levels of IMP after challenge with Streptococcus iniae by intraperitoneal injection (Song et al., 2012).

Similar results are reported in terrestrial livestock as observed in fish. These tendencies are clearly observed in a relatively young animals with a higher Nucleotide requirement. In an infected environments with diarrheal pathogens such as *Escherichia coli* K88, there are reports verifying improving (Increase of beneficial bacteria and reduction of harmful bacteria, diarrhea score) by supplementing Nucleotides in piglet feed (Table 6, Li et al., 2015). Under stressed conditions such as high density or unsanitary environment, the immunity improves significantly with supplementing Nucleotides in poultry feed (Jung and Batal, 2012).

Table 6. The effects of dietary Nucleotide supplementation on fecal microflora of weaned pigs (Li et al., 2015)

Item, log_{10}cfu/g	CON	R150	R220	R275	SEM[b]	Contrast[c]		
						1	2	3
Lactobacillus	6.83	7.08	7.24	7.30	0.14	0.02	0.26	0.76
E.coli	6.54	6.13	5.83	5.41	0.17	0.001	0.01	0.76

[a]Abbreviation: CON basal diet, R150 basal diet + Nucleotides 150 mg/kg, R220 basal diet + Nucleotides 220 mg/kg, R275 basal diet + Nucleotides 275 mg/kg
[b]Standard error mean
[c]Contrast: 1) CON vs. mean of Nucleotide groups; 2) R150 vs. R275; 3) R220 vs. mean of R150 and R275

2.4.3 Improvement in Gut Health

Most of Nucleotides in the feed are ingested in nucleoprotein form. Then, several digestive enzymes break down these into nucleic acids finally into Nucleotides. The majority of them (approximately 90%) are absorbed in the small intestine as nucleotides. The absorbed Nucleotides are degraded or synthesized into Nucleotides or bases (purine/pyrimidine) in small intestine as needed. In short, the small intestine is the most important organ for formation the body's Nucleotide pool. The small intestine store approximately 25-50% of all the absorbed Nucleotides Intestinal epithelial cells go through a rapid cell division (circulation) since they are easily removed and proliferated. It is reasonable to deduce that small intestine is a major organ forming the Nucleotide pool due to its rapid cell replication rate requiring large amounts of Nucleotides.

Diarrhea must be managed well in order to prevent loss of growth performance and increasing mortality. Diarrhea causes not only physical change of feces but also a removal of epithelial cells which eventually lowers an absorption rate of nutrients ingested. This can also lead to remaining nutrients to be utilized as nutritional sources for intestinal

pathogenic microorganisms (e.g. Bacteroides, Clostridium), inducing a continual pathogen load. Today diverse feed additives are being developed to meet farmers' need for prevention and initial treatment of diarrhea. 'Nucleotide' is one of new option. Bifidobacteria, a beneficial intestinal bacteria lowers the pH of intestinal contents by hydrolysis of sugar. Bifidobacteria, has been reported to improve gut health by inhibiting a proliferation and growth of pathogenic microorganisms.

This has been confirmed by in vitro experiments. In which a significant positive correlation between the proliferation of Bifidobacteria and the amount of dietary Nucleotide supplementation. Li et al. (2015) investigated the effects of dietary Nucleotides on piglet after being challenged with *E.coli* K88, and found a tendency decreasing fecal counts of *E.coli* and increasing fecal counts of beneficial Lactobacillus in the Nucleotides supplemented treatments (Fig. 8). The improvement in gut health of piglets decreased diarrhea score with the addition of the Nucleotide in the feed has been reported.

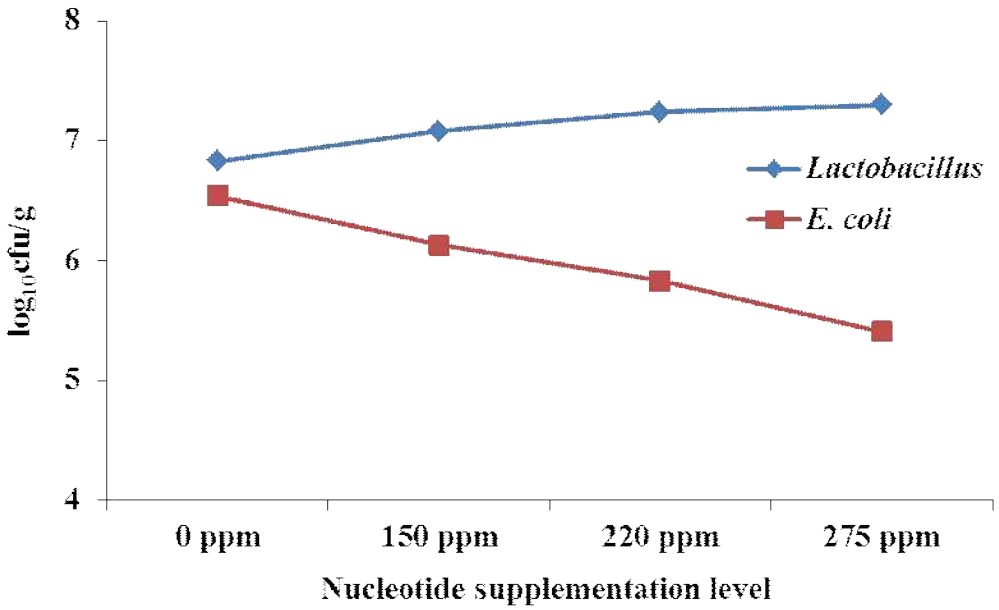

Fig. 8. The effect of dietary Nucleotide supplementation on fecal microflora (Lactobacillus and *E.coli*) of weaned pig (Li et al., 2015).

Recent studies identifying microbiota all microbiomes in intestinal microflora, determined which species are affected by the environment (diet, disease, stress, etc.). Recently, analytical methods like metagenomics are receiving attention by aiding in our understanding of microbial communities and their mutual ecological roles. Recent experiments have confirmed that IMP has improved the effect on atopy in pets (puppy); and it might which may be due in part its effect on the intestinal microorganism.

2.4.4. Nucleotide as Antibiotic Alternative

Since the 1940s, antibiotics have been widely used to improve productivity based on their effectiveness to prevent or suppress livestock diseases, and to promote thus improving growth. However, the worldwide use of antibiotics has been gradually reduced or forbidden due to concerns of antibiotic-resistant bacteria by the their abuse. In South Korea, the number of antibiotics for growth promoter of promotion in compound feeds was reduced from 53 to 25 in 2004. An additional 7 antibiotics have been banned since 2009. It is completely prohibited to use antibiotics except for some anti-coccidial agents since July 2011. This trend is confirmed not only in the livestock industry but also in the aquaculture industry; the use of antibiotics has been significantly reduced in countries with advanced aquaculture technologies such as Europe and Japan.

As a result, there are more and more interests and need for alternative compounds (dietary fiber, beta-glucan, organic acids, etc.) which can effectively replace antibiotics. Studies on the development of functional feed additives have been actively conducted. Zinc oxide (ZnO) one of these alternatives, is a trace mineral frequently added to feed mainly because of its effectiveness in improving the immunity of piglets. However, its use is increasingly regulated due to excessive use of zinc increases the risks of the environmental pollution through feces. As previously mentioned, Nucleotide has been reported to directly or indirectly influence gut health and overall immune function. Many researchers studied nucleotide functionalities in piglet diets with reduced levels of zinc oxide. According to the result of this study indicate when

0.5% of IMP and GMP were added to the feed at 0.05% with 80ppm of zinc oxide, there was no significant difference in the frequency and degree of diarrhea in piglets compared with the control feed containing 500 ppm zinc oxide.

Consequently, Nucleotide acts as an immunostimulant or an immunostimulatory agent, and is expected to which may be used as an alternative to antibiotics based on its immune function enhancement effects.

3. Nucleotide on Piglet Performance

3.1. Effects of Nucleotide on the growth performance of piglet

Many the studies in which dietary nucleotide were supplemented to weaning pigs feed have reported results on improvement of growth performance in pigs. During periods of rapid growth, the need for nucleotide is elevated in immuno-compromised animals. Especially, in newly weaned pigs, those factors are exist so that high amount of nucleotide would be required during the rapid growth (C.D. Mateo and H.H. Stein., 2004). The fact that nucleotide synthesis is composed of glutamine-requiring process and an energy because weaning pigs are normally lack of both energy and glutamine. Also, it could be a reason that pigs are not able to synthesize sufficient quantities of nucleotide in the immediate post-weaning period. In case of, if nucleotide is not supplemented sufficient amount of requirement in weaning pigs, damaged villi while after shifting from milk to solid diet is not able to recover quickly then weaning pigs growth performance will decrease due to lack of nutrient absorption.

In the Weavern and Kim et al. (2014) study, the growth performance of the one treatment with nucleotide supplementation was having effect on the performance. The experiment consisted of four different diet : A (control), 0.02% nucleotide; B, 0.05% nucleotide; C, 0.1% nucleotide; D. As a result, during phase 1, there was a linear increase in BW ($P = 0.008$), ADG ($P = 0.007$), ADFI ($P = 0.005$), and G:F ($P = 0.015$) as dietary nucleotides increased from 0.0 to 1.0 g/kg. During phase 2, growth performance traits were not altered from d 7 to 14, but ADG increased during d 14 to 21 and tended to increase from d 21 to 28 ($P = 0.043$ and $P = 0.053$, respectively). Throughout phase 2, ADFI increased ($P < 0.001$) as nucleotide supplementation increased. Throughout the entire study, the ADG and ADFI increased ($P = 0.031$ and $P < 0.001$, respectively) with increasing nucleotide supplementation to the diet.

These variations may connect closely to the type and levels of nucleotide supplemented in the diet. Zomborszky-Kovács et al. (2000) found that supplementation of the synthetic nucleotide bases adenine and uracil at 0.5 g/kg to weanling pig diets improved growth performance. Sauer et al. (2012) found that feeding a mixture of pure nucleotides (5′ AMP, 5′CMP, 5′GMP, 5′IMP, and 5′UMP) to weanling pigs increased ADFI but did affect to improve gain or feed efficiency. However, another study showed that a mixture of nucleotides added to the diet at 0.1 g/kg did not improve weanling pig growth performance, which was speculated to have occurred due to an insufficient dosage of nucleotide (Lee et al., 2007).

3.2. Effects of Nucleotide on immune system and intestinal morphology of piglet

Nucleotides are building blocks of DNA and RNA and serve to carry packets of energy withing the cell, play an important role in metabolism and act as co-factors in enzymatic reactions (Kempeneers., 2003). As a result of banning AGP in feed in the EU, the selection of new pig production system such as improving immune system, healthy and fast growing animals is a fundamental step to regulate the current production system (P. Superchi et al., 2011). Dietary nucleotide have been researched to enhance immune responses in weaning pigs. A higher number of intraepithelial lymphocytes and macrophages in the ileal tissues of piglets receiving dietary nucleotides after weaning have a large number of macrophages and intraepithelial lymphocytes in the ileal tissues (Domeneghini et al.. 2004). Šperanda et al. (2008) reported an immunostimulatory effect of NRYE (Nucleotide-rich Yeast Extract) regarding to increasing the percentage of lymphocytes, especially CD4 and CD8 subpopulation of T cells. Also, IgA levels in plasma and bile may be increased when nucleotides supplemented in the diets (Lee et al., 2007; Sauer et al., 2012a). In above case, a high concentration of IgA in the intestinal mucosa may indicate stronger humoral mucosal immunity (Macpherson et al., 2008). There is an experiment that supplementing 0.075 and 0.1% of nucleotides in piglets diet resulted a reduction in the mortality rate and diarrhea incidence that was associated with a beta-haemolytic strain of *E.coli* (Martinez-Puig et al., 2007).

Additionally, positive effects of dietary nucleotides have been reported in many studies using disease challenge experiments (Samuel Mwangi., 2016). For instance, under challenged condition, dietary nucleotides supplementation decreased the concentration of IL-6 after 2 and 4 hours and serum TNF-α, respectively, following a 50 μg/kg body weight of *E.coli* lipopolysaccharide injection to piglets, which suggests a stimulation of macrophage and B cell proliferation, respectively (Hung., 2015; Li et al., 2015).

Ⅲ. Effect of Different Type of Nucleotide Supplementation on Growth Performance, Blood Profile, Diarrhea Score and *E.coli* Challenge in Weaning Pig Diets.

Abstract: Two experiments were conducted to evaluate the effect of different type of nucleotide supplementation on growth performance, blood profile, diarrhea score in weaning pigs (Exp. 1) and effect of IMP and GMP use on anatomic trait and diarrhea of weaning pigs under *E.coli* challenge (Exp. 2). In Exp.1, a total of 288 crossbred ([Yorkshire x Landrace]) x Duroc) pigs (7.17 ± 1.176kg BW) were assigned six treatments considering sex and initial body weight in 6 replication with 8 pigs per pen in randomized complete block (RCB) design. Those pigs were weaned at 42 days(Phase 1. 0~21days, Phase 2. 21~42days) of age and fed with a diet supplemented with CON: Corn-soybean meal basial diet + ZnO 500 (Phase I) / 300ppm (Phase II); I5: Corn-soybean meal basial diet + IMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); I10: Corn-soybean meal basial diet + IMP 0.10% + ZnO 500 (Phase I) / 300ppm (Phase II); IG2.5: Corn-soybean meal basial diet + IMP 0.025% + GMP 0.025% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5: Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5a: Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm (Phase I / Phase II). In Exp

2. total of 45 crossbred ([Yorkshire x Landrace]) x Duroc) pigs (8.84 ± 0.20kg BW) were assigned five treatments considering sex and initial body weight in 1 replication with 9 pigs per pen in randomized complete block (RCB) design. In case of Exp. 2, it was conducted same design with Exp. 1 but control was removed and weaning pigs were orally challenged with *E.coli* K88 on 14 days of age. In the Exp. 1, I5 and IG2.5 showed the best growth performance among treatments. Also, IG2.5 especially improved ADG during each phase and increased BW significantly during each phase. In blood profiles, there was no significant difference in IgG, IgA, IgM, TNF-α and among treatments. Likewise, there was no crucial difference on diarrhea incidence during each phase. In the Exp. 2, no significant difference was observed on 14 and 15 days of age in length of intestine but there was critical difference with duodenum and ilium in IG2.5 and IG5a on 42 days of age. In addition, morphological changes of the small intestine including longer villus height and crypt depth compared to other treatments in general was highly affected by IG5a to show the most positive effects on nutrient absorption and utilization. Like Exp.1, there was no significant difference in diarrhea incidence and score but it shows that nucleotide supplement would be an alternative additive of zinc oxide for preventing diarrhea in weaning.

Keywords : Nucleotide, IMP (Inosine Mono-Phosphate), GMP (Guanosine Mono-Phosphate), Weaning pig, Growth performance, Intestinal morphology, *E.coli* K88

Introduction

Weaning stress is one of the most stressful period for pigs (Weaver and Kim, 2014). During this particular process, an ultimate goal of commercial farms is having sufficient growth performance in any kinds of challenged circumstances to avoid damages economically (Pluske et al., 1997; P. Superchi et al., 2011; Campbell et al., 2013). Since a weaning stress causes a retardation in growth with low feed intake and 60-70% decreased energy intake, stress management in weaning period is significant. Additionally, weaning stress also impairs mucosal barrier function of porcine intestine so that it can bring about diarrhea and dehydration which lead to increasing mortality rate (Smith et al., 2010; Hong et al., 2012). To solve these problems, adding AGP (antibiotic growth promoter) for the feed has been widely used for improving promoting animal growth, feed efficiency, decreasing incidence of disease and diarrhea, and enhancing the quality of the animal products (Verstegen and Williams., 2002; Cheng et al., 2014). However, AGPs have been prohibited in 1986 from Sweden for the first time in the world and forbidden in the EU in 2006 and in Korea in 2011 , gradually because antibiotic resistant-bacteria were found in humans and livestock (Castanon, 2007; Cogliani et al., 2011). According to the historical background, many kinds of antibiotics alternatives have been studied such as enzymes, probiotics, prebiotics, plant extracts, and zinc to prevent increasing mortality rate and damaged to commercial farms (Partanen and Mroz, 1999; Millet and Maertens, 2011; Seal et al., 2013).

Nucleotide is one of these immune enhancers, largely divided into

IMP, GMP which is purine, CMP, TMP and UMP which is pyrimidine. Nucleotide is the main constituent of the cell nucleus and is involved in most metabolism in the cell and plays an important role as a structural, metabolic, and physiological regulator (Lee et al., 2007). IMP and GMP, which belong to purine, are characterized by promoting the development and division of cells in the fetal (Uauy et al., 1994) and growing stages and enhancing cellular immunity (Hendrick and Ryan., 1970; Carver et al., 1994). Purine nucleotides are reported to consume more energy and time when synthesized in the body than pyrimidine nucleotides (Kornberg et al., 1954). Furthermore, the addition of nucleotide in nursery feeds has been reported to increase the weight of the small intestine (Lee et al., 2007), and studies have shown that the *de novo* synthesis of the nucleotide in the body does not meet the requirement for intestinal cells (Uauy et al., 1994; A. Gil, 2002). Domeneghini (2004) also reported that the addition of 500mg/kg of nucleotide in the feed increased the morphological development of the small intestine. Several previous studies (Danilova et al., 1999; Weaver and Kim, 2014) have been carried out to identify the effect of nucleotide on the growth performance of piglets by using it as an additive to increase immunity and improve intestinal health, however, research has not yet been conducted on whether IMP or GMP is more effective, or a performance that occurs when a mixture of these is used.

Therefore, this study was carried out to investigate the effect of various kinds of nucleotide in feed on the anatomical characteristics in a challenged circumstance, incidence of diarrhea of piglets for the improvement of immunity, growth performance of piglets and to investigate the optimal addition of nucleotide.

Materials and Methods

Experimental animals and management

Exp. 1 : A total of 288 crossbred ([Yorkshire x Landrace]) x Duroc) pigs (7.17 ± 1.176kg BW) were assigned six treatments considering sex and initial body weight in 6 replication with 8 pigs per pen in randomized complete block (RCB) design. Exp. 2 : A total of 45 crossbred ([Yorkshire x Landrace]) x Duroc) pigs (8.84 ± 0.20kg BW) were assigned with 5 treatments considering sex and initial body weight in 1 replication with 9 pigs per pen in randomized complete block (RCB) design. Both experiments pen were fully-concrete floor facility (1.54 × 1.96 m^2) in experimental period and equipped with feeder, water nipple, and environmentally controlled facility in Seoul National University Farm. The experimental periods were 6 weeks respectively. Experimental period consisted of 2 phases, phase 1 was 0-3 week and phase 2 was 4-6 week. Body weight, and feed intake were collected at the end of each phase in order to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F ratio). In addition, feeding to all piglets were recorded each day, and waste feed in feeder was recorded on the end of each phase.

Experimental design and diet

Exp.1 : Experimental pigs were allotted with randomized complete block (RCB) design. The first factor was four levels of IMP and IMP + GMP (IMP 0.05%, IMP 0.10%, IMP 0.025% + GMP 0.025%, IMP 0.05% + GMP 0.05%). The second factor was three levels of zinc (Phase 1 :

500ppm, Phase 2 : 300ppm, IMP 0.05% + GMP 0.05% treatments with zinc 80ppm). Dietary treatments included : 1) CON (Corn-SBM based diet + ZnO 500 / 300ppm), 2) I5 (Corn-SBM based diet + IMP 0.05% + ZnO 500 / 300ppm), 3) I10 (Corn-SBM based diet + IMP 0.1% + ZnO 500 / 300ppm), 4) IG2.5 (Corn-SBM based diet + IMP 0.025% + GMP 0.025% + ZnO 500 / 300ppm), 5) IG5 (Corn-SBM based diet + IMP 0.05% + GMP 0.05% + ZnO 500 / 300ppm), 6) IG5a (Corn-SBM based diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm).

Exp.2 : Experimental pigs were allotted with randomized complete block (RCB) design. The first factor was four levels of IMP and IMP + GMP (IMP 0.05%, IMP 0.10%, IMP 0.025% + GMP 0.025%, IMP 0.05% + GMP 0.05%). The second factor was three levels of zinc (Phase 1 : 500ppm, Phase 2 : 300ppm, IMP 0.05% + GMP 0.05% treatments with zinc 80ppm). Dietary treatments included : 1) I5 (Corn-SBM based diet + IMP 0.05% + ZnO 500 / 300ppm), 2) I10 (Corn-SBM based diet + IMP 0.1% + ZnO 500 / 300ppm), 3) IG2.5 (Corn-SBM based diet + IMP 0.025% + GMP 0.025% + ZnO 500 / 300ppm), 4) IG5 (Corn-SBM based diet + IMP 0.05% + GMP 0.05% + ZnO 500 / 300ppm), 5) IG5a (Corn-SBM based diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm). Experimental diets were formulated for 2 phases, including weaning phase 1 (0-3 week) and weaning phase 2 (4-6 week). All nutrients of experimental diets except CP and ME were met or exceeded the nutrient requirement of NRC (1998). Formula and chemical composition of experimental diet were presented in Tables 1, 2 (Exp. 1) and Table 6, 7 (Exp. 2).

Blood sampling and analyses

In case of ExP. 1, blood samples were taken from the jugular vein of five pigs near average body weight in each treatment for measuring IgA, IgG, IgM, TNF-α, IL-6 when the body weights were recorded. Collected blood samples were centrifuged for 15 min at 3,000 rpm on 4 ℃ (Eppendorf centrifuge 5810R, Germany). The sera were carefully transferred to 1.5 ml plastic tubes (Serum tubes, BD vacutainer® SSTTM II advance, UK) and stored at –20 ℃ until analysis. To investigate the degree of TNF-α, IL-6 was analyzed using an ELISA assay (Microplate reader, VERSA Max, Quantikine porcine TNF-α/ TNFSF2 Immunoassay / Porcine IL-6 Immunoassay, MN, USA) and IgA, IgG, IgM concentration was measured by an TIA assay (Modular analytics, P, Tina-quant IgA/G/M Gen.2 kit, Roche, Germany) to determine immune status.

E.coli challenge

In case of Exp. 2, the attack was carried out on the 14th day after the induction, and *Escherichia coli* O8: K88 from the KVCC (Korean Veterinary Culture Collection) was distributed. The inoculation was carried out by diluted *E.coli* through piglets orally. Data were collected on day 14, day 15, and day 42 of the experiment.

Weight of body and digestion organ

In case of Exp. 2, on the 14th, 15th, and 42nd day after weaning, 3 pigs of each time were selected and slaughtered so as not to exceed the average weight of the treatments at each time point. The body weight of the slaughtered pigs was measured, and the digestive organs were extracted

from the anatomy, and small intestine weights, large intestine weights, spleen weights, and liver weights were measured.

Morphology of small intestine

In case of Exp. 2, small intestine was removed from piglets slaughtered on days 14, 15 and 42 after weaning. For the histological examination after slaughter, 10 cm of the proximal duodenum, jejunum, ileum of the small intestine were collected. After removal of the digest, samples from the middle of each duodenum, jejunum, and ileum tissue were cut 6-8 cm, cleaned and stored in neutral-buffered formalin solution until further morphological analysis. The samples were then cut into two parts at each segment for cross and length section of intestine surface and then processed by the standard by the standard paraffin method. Sections (2~3cm) were stained with haematoxylin and eosin, and examined under a light microscope. It was used a Leica DM500 microscope with Leica DFC425 coupled to the computer program (Leica Application suite software) for image analysis. The villus height was measured as the distance between the crypt mouth and the tip of villi. The crypt depth was measured as the distance between the basement membrane and the mouth the crypt. The villi and crypts were expressed in um (micrometer) units. In case of tissue photographs, small intestine days 14 and 15 after weaning was photographed 100 times and the small intestine days 42 after weaning was photographed 40 times.

Incidence of Diarrhea

In case of Exp. 1, observation of diarrhea incidence was conducted every 8:00 am for 42 days. The diarrhea index was measured once a day for 8 piglets in each pound and the number of diarrhea was counted and scored. Measurements of all diarrhea indexes were performed by one representative to maintain maximum objectivity.

In case of Exp. 2, observation of diarrhea incidence was conducted every 8:00 am for 42 days. The number of all the piglets showing the presence of hydrosoluble diarrhea around the anus was divided by the total number of piglets. In the case of diarrhea, the score was recorded according to the degree of the completely solid state feces as 0 and the completely liquid state feces as 4.

Statistical analysis

In case of Exp. 1, Statistical analysis was performed using the MIXED procedure of SAS Institute Inc., Cary, NC, USA. For the addition of nucleotide levels (CON, I5, I10; and CON, IG2.5, IG5), the SAS General Linear Model was used and the IG5 and IG5a treatments were tested for the significance of data collected using the SAS T-test Model. Through this analysis, growth performance, blood characteristics and frequency of diarrhea were analyzed. In the case of growth performance and diarrhea, the experimental data was set as the experimental unit and in case of blood profile the experimental data was set as the experimental unit. Also, in case of significant difference, it is presented with $P < 0.05$ and $P < 0.01$ means highly significant difference and $0.05 \leq P < 0.10$ shows that it has a tendency.

In case of Exp. 2, Statistical analysis was performed by using the general linear model (GLM) of SAS, and the results were compared by the least significant difference (LSD) multiple test method (I5 − IG5a). In addition, it progressed to 2 × 2 factorial of additive factor (IMP / IMP + GMP) × addition factor (0.05 / 0.10%) (I5 − IG5). All statistical analyzes showed a significant difference for $P < 0.05$, with a significant difference for $P < 0.01$.

Results and Discussion

Growth performance − Exp. 1

Table 3 shows the growth performance of the treatments of nucleotide addition test in the nursery feed. There was no statistically significant difference between the treatments in body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed ratio (G: F ratio). However, in phase 2, there was a significant difference in each treatment in ADFI and the control was significantly lower than the other treatments ($P < 0.05$). There was also no statistically significant difference in feed intake and feed conversion ratio among the treatments for 6 weeks, and IG5a was the lowest in BW ($P = 0.095$) and daily weight gain ($P = 0.099$). Various causes such as decreased growth retardation and feed intake occur due to various stresses after weaning in weaned piglets (Kim et al., 2010). In conjunction with this increase in stress, the demand for nucleotide is further increased. However, additional nucleotide feeding is required because weaning pigs are isolated from sows and the possibility of feeding nucleotide from sow milk is lost (Mateo et al., 2004). In addition, there is a precedent study in which the addition of nucleotide in the feed has a positive effect on increasing the feed intake of the weaning pigs (Kubitza et al., 1997). At this point, purine-based IMP (Disodium 5'-Inosinate) and GMP (Disodium 5'-Guanylate) are included in the feed. According to a previous study, the amount of nucleotide added was increased linearly according to the situation. As the amount of nucleotide added increased, daily gain of body weight and daily feed intake increased (Weaver and Kim., 2014). The results of this

experiment showed that there was no significant difference in the growth of the piglets in phase 1, but the ADFI of the piglets was significantly different (P <0.05) in phase 2 and CON showed the lowest value. During the 42 days total experimental period, the treatments showed significant differences in body weight (BW) and body weight gain (ADG), and the values of the addition of nucleotide and 500 ppm of zinc oxide treatments were higher than those of CON treatment.

In conclusion, the addition of nucleotide in the feed for weaning pigs have a positive effect on the growth performance of the weaning pigs especially the treatments I5 (IMP 0.05%) and IG2.5 (IMP 0.025% + GMP 0.025%) promotes growth performance.

Blood profiles − Exp. 1

The effect of nucleotide on blood profiles was presented in Table 4. As a result of the analysis, there was no statistically significant difference in blood profiles (IgG, IgA, IgM, TNF-α and IL-6) during the whole experimental period.

For piglets, the major nutrient intake from birth is from colostrum and breast milk (Farmer et al., 2009). Among the components of colostrum or breast milk, immunoglobulin-related proteins are divided into two groups: first, casein and whey protein (Pond, 1973). Among whey proteins, albumin, α-lactoalbumin, β-lactoglobulin, immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), lactoferrin and other minor proteins are contained in the blood (Pond, 1973). IgG is the most abundant part of colostrum and its content is known to be 76%. In addition, there is a precedent study in which the IgG content in colostrum

is highest for 24 hours postpartum and this has a positive effect on the growth of the weaning pigs (Devillers et al., 2011). However, as colostrum is passed from milk to colostrum, IgG is reduced by approximately 85% and IgA is the highest in breast milk (Klobasa et al., 1987). In addition, IgA and IgM play an important role in the protective role of intestinal mucosa in piglets and become the most abundant immunoglobulins in breast milk 24 hours after piglets are born. (Brandtzaeg, 2003). In the case of nucleotide, adding nucleotide in the feed has been reported to increase the serum IgA content of 28-day-old weaning pigs (Lee et al., 2007). In addition, serum IgA concentrations were also increased when nucleotide were added via water rather than feed (Sauer et al., 2012). In another previous study, these results may be due to differences in the types and amounts of nucleotide added to feeds or differences in treatments (Hung, 2015). In this experiment, it is considered that the addition level of nucleotide does not affect with IgG, IgA, IgM. Zinc oxide has been reported to play an important role in activating more than 300 enzymes (Chasapis et al., 2012) and also plays an crucial role in immunity, as trace minerals used in various applications (Fraker et al., 2000). There is also a precedent that zinc oxide has a positive effect on increasing blood immunoglobulin concentration (Wang et al., 2011). In a previous study, the level of zinc oxide in feed was 80 ppm per kilogram, but there was no change in blood immunoglobulin concentration (Castillo et al., 2008). The results of this experiment showed no significant difference between IG5 and IG5a treatments. This suggests that even if the level of zinc oxide is lowered to 80 ppm, it does not negatively affect the blood immunoglobulin concentration when nucleotide added.

TNF-α is an indicator of rapid response to infection and inflammation in the body (Desch et al., 1989; Bemelmans et al., 1996). The amount of TNF-α secreted in the blood is associated with infection of different types of bacterial or viral diseases, among which *E.coli* is a well-known pathogen. In the nutritional insufficiency, the secretion of TNF-α is reduced and the infection of Gram-positive bacteria is induced (Doherty et al., 1994). Piglets are frequently exposed to Gram-positive bacteria such as *E.coli* for reasons of infection. One of the main causes is the poor digestion and absorption due to the degradation of digestive function due to changes in feed form and environment. In addition, there is a precedent study in which the amount of TNF-α secretion decreases as a result of various stresses during the process (Kusnecov et al., 2002). In the case of nucleotide, there is a precedent study that it is helpful to activate immunoglobulin or T-cell related to blood immunity when it is added in feed (Lee et al., 2007). This suggests that nucleotide not only helps to improve T-cell function but also assists pigs (Cameron et al., 2001). Nucleotide is also known to help macrophage activation of lymphocytes in intestinal and intravenous epithelium (Domeneghini et al., 2004). In the case of the present experiment, during the 42 days experimental period, no significant difference in the treatment of TNF-α was observed in the addition of the nucleotide so that adding nucleotide have no negative effect on TNF-α secretion.

In the case of zinc oxide, it regulates the intensity and duration of the immune response and is involved in the cytokine protein secretion involved in cell-to-cell transmission (Von Bülow et al., 2007). In addition, there is a precedent study that induces mRNA expression of TNF-α and

also helps secretion in the body (Wellinghausen et al., 1996). In this experiment, there was no significant difference in treatments according to the addition level of zinc oxide in the feed. This suggests that even if the level of zinc oxide is lowered to 80 ppm, it does not negatively affect TNF-α secretion in the body.

IL-6 is known to play a major role in bacterial infection (Song et al., 2005). It also plays an important role in maintaining cell integrity (Li et al., 2007a). Changes in intestinal cytokines are caused by: First, sudden changes in the shape and environment of the feed cause morphological and functional changes of the small intestine. Second, there is an increase in T-cell, B-cell and macrophage in the mucosa of the small intestine (Pič et al., 2004; Wu, 1995; 1996). Especially IL-6 has various functions that it is essential to divide B-cell and play a role in the activation of T-cell receptors and helps to switch from T-cell into IL-2 (Davis et al., 2002).

Nucleotide is known to mediate T-cell mediated responses in the immune system (Shinya et al., 1997). In addition, a previous study has shown that nucleotide enhances the response in the body's immune response (Jyonouchi, 1994). Also, there is a precedent research that stimulates the secretion of interlukin (Shinya et al., 1997). The addition of nucleotide to feeds has been shown to help interleukin to counteract the effects of milk allergy (Shinya et al., 1997). In this experiment, it is considered that the addition of nucleotide in the feed does not have a negative effect on the immune response in interlukin measurement.

In the case of zinc oxide, it plays an important role in the immune system in the body and is known to help for making antigens and increase the antigen in the body (Roy et al., 2013). In this experiment,

there was no significant difference in the treatment of interlukin. Additionally, when IG5 and IG5a treatments were compared, it was concluded that the addition of zinc oxide to 80 ppm did not have any negative effects.

In conclusion, the addition of nucleotide in the feed did not cause any negative effects on blood immunoglobulin (IgG, IgA, IgM), TNF-α and IL-6 even with IG2.5(IMP 0.025% + GMP 0.025%). Finally, it is considered that even if the addition level of zinc is lowered to 80 ppm, there is no negative influence on blood profile.

Weight of internal organ – Exp. 2

The effect of addition of nucleotide on the weight of organs in nursery feed is shown in Tables 8 and 9. There were no significant differences in the BW, total internal organs weights, small intestinal weights, large intestinal weights and liver weights (P> 0.05). And, no significant difference was found between the factors depending on the kinds and amounts of the nucleotide and the interaction between them in each item (P> 0.05).

There was no major difference in the spleen weight between days 14 and 42 (P> 0.05), but no significant difference was found between the factors (P> 0.05) I5 treatment was significantly higher than other treatments (P <0.05) at 15 days' weight (P <0.05).

Lee et al. (2007) reported that the addition of nucleotide in a diet increased the intestinal weight, while Carver (1996) found that the addition of nucleotide in feed significantly contributed to the growth and differentiation of digestive organs. However, there was no significant

difference in total small intestine weight, small intestine weight, and large intestine weight in this experiment.

According to Heppel et al. (1955), ribonuclease and spleen phosphodiesterase catalyze the nucleotide exchange reaction in the synthesis of nucleotide conjugates. In the glucocorticoid action, the purine-based nucleotide synthesis reaction is also influenced by liver and spleen antagonism (Feigelson and Feigelson, 1966). Thus, it appears that there is a functional link between the purine and the spleen, and purine is known to be highly active in mammalian testis and spleen (Itoh et al., 1992).

The spleen is the most important lymphatic organ in the animal body, accounting for about 25% of the total lymphatic organ weight. The spleen may become larger in response to infection or inflammation, which is considered to be due to an increase in acute inflammatory reactants (Mireles et al., 2005). There was no difference in the liver weight in this experiment, but a significant difference was found in the 15 day spleen weight after weaning. It was concluded that *E.coli* challenge on day 14 induces intestinal infections and inflammatory responses and this immune response is thought to increase the expression of acute immune protein in the spleen and increase the weight of the spleen. It was concluded that the addition of IMP 0.05%(I5) significantly increased infection and inflammation compared to other treatments. On the other hand, the development of small intestinal epithelial cells and mucous membranes, which play a primary protective role against external invasion factors, are thought to reduce the production of acute phase proteins by inhibiting the body infection. In addition, the difference in the nucleotide types was significant, suggesting that the use of IMP + GMP in combination is

better rather then using IMP alone improved the ability of the small intestine to defend.

As a result, the addition of nucleotide and the control of addition amount did not affect the weight of the small intestine, colon and liver. However, in spleen weight, the use of IMP + GMP in combination has better result than the use of IMP alone to improve small intestine. Additionally, no significant difference was observed in other treatments even when ZnO in feed was decreased in all items. It is expected that nucleotide can replace ZnO in feed.

Length of intestine – Exp. 2

Table 10 shows the effect of addition of nucleotide on the small intestine length in the nursery feed. There was no significant difference in the length of the small intestine between 14 and 15 days of treatment due to differences in treatments, factors, or relationship between each factor ($P > 0.05$). However, there was a significant difference in the 42-day-old duodenum and in the jejunum by nucleotide ($P < 0.05$) that combination with IMP + GMP has better result on intestine length. Especially in duodenum, there was significant difference between treatments ($P < 0.05$) and IG2.5 and IG5a treatments were significantly longer than other treatments.

Kubota (1966) reported that nucleotides are rapidly absorbed and used in young animals immediately than in adult animals, suggesting that this may lead to growth promoting effects (Gyorgy, 1971). As mentioned earlier, nucleotides have a positive effect on the development of the small intestine, and therefore, it is expected to have a positive role in the length

of the small intestine and the length of the small intestine.

In this experiment, it was statistically significant that using IMP + GMP mixed material increased the intestine length rather than using IMP alone on 42 day. Although it cannot be explained the exact mechanism, IMP is able to catalyze the activity of GMP-reductase, which regulates the balance of intracellular GMP and AMP (Kokunin et al., 1987). The decrease in ZnO in feed also had a positive effect on the length of the duodenum.

Therefore, in the length of the small intestine, it has a better result in IG2.5 rather than using IMP alone, which is considered to be a substitute for ZnO.

Intestine morphology

The effects of the addition nucleotide on villus height and crypt depth in the duodenum, the jejunum and the ileum are shown in tables 11, 12 and 13. In the villus height and the crypt depth of the duodenum, there were a significant difference ($P<0.05$) and highly significant difference ($P<0.01$) on IG5a treatment with lower ZnO addition and the significantly higher value in the villus height and the crypt depth ratio ($P<0.05$). In the crypt depth of the jejunum, IG2.5 treatment was significantly increased at 15 days after weaning ($P <0.05$), but the difference was not significant. In the ileum, there was a significant difference in the villus height and the crypt depth at the 42 days ($P <0.05$) with IG2.5, and the villus height increased and the crypt depth decreased. When compared with other treatments, IG5a treatment showed in the crypt depth with significant difference ($P <0.01$) and IG5a also

showed significantly higher result ($P < 0.05$) in the villus height and the crypt depth ratio. In addition, I10 and IG5 treatments treated with 0.10% of nucleotide showed a significantly lower value than those of the other treatments in the crypt depth at 42 days of age and showed the highest values at I5 treated with IMP alone ($P < 0.01$).

Nunez et al. (1990) have shown that when various nucleotide, including IMP and GMP, are added, they increase mucosal immunity and enhance the resilience of intestinal walls by increasing intestinal mucosal proteins. Domeneghini et al. (2004) also showed that the addition of nucleotide increased the morphology of the small intestine by increasing the villus height, while increasing the crypt depth. Based on these previous studies, it was concluded that the morphology improvement of the jejunum and the ileum in this experiment was influenced by the recovery effect of the nucleotide and that the effect of mixed IMP + GMP was better than using IMP alone. When IG5a treatment was compared with other treatments, the use of nucleotide showed a positive result even when ZnO content was lowered. In the intestinal morphological changes, the nucleotide is expected to play a role as a substitute for ZnO.

Therefore, the use of IMP + GMP in the morphology of the small intestine wall can prevent damage after *E.coli* challenge and to help the recovery, and the use of nucleotide may be a substitute for the use of ZnO in the feed.

Incidence of diarrhea

Exp. 1 : Table 14 shows the incidence of diarrhea of piglets when nucleotide is added to nursery feeds. The incidence of diarrhea, the whole

experimental period showed significant differences between each treatment ($P < 0.01$; $P < 0.01$; $P < 0.01$). Although IG5a treatment showed the highest diarrhea frequency compared to other treatments, the addition of nucleotide did not affect the diarrhea index of piglets.

In the case of weaning pigs, various types of stresses are experienced. In this process, digestive organs go through the adaptation period mainly due to the ingestion of new type of feed, ie, solid feed (Salobir et al., 2005). In particular, changes in the villus height and crypt depth lead to poor nutrient and digestive capacity from feed intake (Vondruskova et al., 2010). These changes result in decreased immunity and viral, bacterial and parasitic diarrhea (Lalles, 1993). As the frequency of diarrhea increases, the rate of growth decreases, and in severe cases, it continues to death (Vondruskova et al., 2010). As shown in these results, diarrhea decreases in average daily gain (ADG) and in daily feed intake (ADFI) have a negative impact on the growth performance. In this experiment, it was shown that the addition of nucleotide in the feed has no effect on reducing the diarrhea index of weaned piglets.

Exp. 2 (Table 14) : According to Carver (1994), purine-based nucleotide enhances cell-mediated immunity, which is consistent with previous studies (Nunez et al., 1992) in that the addition of nucleotide increases mucosal immunity by increasing intestinal mucosal proteins. This indicates that there is no significant difference between the IG5 treatment with ZnO and the IG5a treatment without ZnO treatment. As a result, the addition of nucleotide shows the effect of preventing diarrhea in place of ZnO have. As a result of this experiment, it can be concluded that nucleotide can prevent diarrhea by replacing ZnO. Therefore, the use of

IMP + GMP in the morphology of the small intestine wall can prevent damage after weaning and to help the recovery, and the use of nucleotide may be a substitute for the use of ZnO in the feed.

Conclusion

Exp. 1 : Addition of the nucleotide in the nursery feed promoted the growth performance and did not affect the blood profile and diarrhea frequency. In conclusion, the addition of nucleotide IMP 0.025% + GMP 0.025% and IMP 0.05% in the nursery diet improves the feed intake and growth performance, and there is no negative influence on the blood profile and diarrhea frequency of zinc oxide reduction.

Exp. 2 : This study was carried out to investigate the effect of the addition of nucleotide on the anatomical characteristics and diarrhea of piglets in piglets. I5 treatment of 15 day spleen weights was significantly higher than other treatments ($P < 0.05$), indicating a significant difference according to the type of nucleotide ($P < 0.05$). Therefore, it is considered that the use of IMP + GMP mixed rather than the use of IMP alone has a positive effect on improvement of small intestine development and defense ability, and it is expected that nucleotide can replace ZnO in feed.

There was a significant difference ($P < 0.05$) between intestinal length and 42 days duodenum and jejunum by the type of nucleotide ($P < 0.05$). Especially in duodenum, there was significant difference between treatments ($P < 0.05$) and IG2.5 and IG5a treatments were significantly longer than other treatments. Therefore, it is considered better to mix IMP + GMP than the use of IMP alone in the length of the small intestine, which is considered to be a substitute for ZnO.

The morphological changes of the small intestine showed a significant difference ($P < 0.01$) and a highly significant difference ($P < 0.01$) in the villus height and the crypt depth of the duodenum in the IG5a treated

with lower ZnO supplementation. And, in the ratio of villus height and the crypt depth, it has a significant difference ($P < 0.05$). In the jejunum, IMP + GMP were significantly increased by the nucleotide at 15 days after weaning ($P < 0.05$), but the difference was not significant. There was a significant difference ($P < 0.05$) between the villus height and the crypt depth at the 42 days in the ileum. When compared with other treatments, IG5a treatment showed a highly significant difference ($P < 0.01$) in the crypt depth between all treatments and the ratio of villus height and the crypt depth IG5a treatment was significantly higher ($P < 0.05$). Therefore, the use of IMP + GMP in the morphology of the small intestine wall can prevent damage after weaning stress and to help the recovery, and the use of nucleotide may be a substitute for the use of ZnO in the feed. Also, no significant differences were found ($P > 0.05$) in incidence of diarrhea. Therefore, it was found that the content of ZnO and when it turned out that there was no problem even when ZnO dropped.

In conclusion, it seems that ZnO can be substituted for nucleotide in weaning pigs, and it is considered to be more advantageous to use the mixture of IMP + GMP in consideration of the development of the digestive tract and the morphology of the small intestine wall.

Table 1. Formula and chemical composition of weaning phase 1 (0-3 week)

Criteria	Treatment[1]					
	CON	I5	I10	IG2.5	IG5	IG5a
Ingredients, %						
Expanding corn	33.79	33.70	33.61	33.70	33.61	33.69
Soybean meal	18.99	19.00	19.01	19.00	19.01	19.00
Soy-oil	0.07	0.10	0.13	0.10	0.13	0.10
Fish meal	4.00	4.00	4.00	4.00	4.00	4.00
Soytide	8.50	8.50	8.50	8.50	8.50	8.50
Barley	18.00	18.00	18.00	18.00	18.00	18.00
Lactose	14.00	14.00	14.00	14.00	14.00	14.00
L-lysine-HCl, 78%	0.27	0.27	0.27	0.27	0.27	0.27
L-methionine, 99%	0.02	0.02	0.02	0.02	0.02	0.02
L-threonine, 99%	0.07	0.07	0.07	0.07	0.07	0.07
Mono-dicalcium phospate	1.17	1.16	1.15	1.15	1.15	1.15
Limestone	0.89	0.89	0.90	0.90	0.90	0.90
Vitamin Mix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Mineral Mix[3]	0.10	0.10	0.10	0.10	0.10	0.10
ZnO (77.3%)	0.05	0.05	0.05	0.05	0.05	0.008
IMP	0.00	0.05	0.10	0.025	0.05	0.05
GMP	0.00	0.00	0.00	0.025	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition[4]						
MaintenanceEnergy, kcal/kg	3,300	3,300	3,300	3,300	3,300	3,300
Crude protein, %	20.56	20.56	20.56	20.56	20.56	20.56
Lysine[5], %	1.35	1.35	1.35	1.35	1.35	1.35
Methionine[5], %	0.35	0.35	0.35	0.35	0.35	0.35
Threonine[5], %	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
Total Phospate, %	0.65	0.65	0.65	0.65	0.65	0.65

[1] CON: Corn-soybean meal basial diet + ZnO 500 (Phase I) / 300ppm (Phase II); I5: Corn-soybean meal basial diet + IMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); I10: Corn-soybean meal basial diet + IMP 0.10% + ZnO 500 (Phase I) / 300ppm (Phase II); IG2.5: Corn-soybean meal basial diet + IMP 0.025% + GMP 0.025% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5: Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5a: Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm (Phase I / Phase II).

[2] Provided per kg of diet : vitamins per kg of complete diets: vitamin A, 8,000 IU; vivitamin D3,1,800IU;vitaminE,60IU;vitaminK3,2mg;Thiamine,2.00mg;riboflavin,7.0mg;pantothenicacid,25mg;niacin,27mg;pyridoxine,3mg;d-biotin,0.2mg;folicacid,1mg;vitaminB12,0.03mg;

[3] Provided per kg of diet: mineral per kg of complete diet: Se, 0.3 mg; I, 1 mg; Mn, 51.6 mg; Cu, 105 mg; Fe, 150 mg; Zn, 72 mg; Co, 0.5 mg.

[4] Calculated value.

[5] Value means total amino acid (%).

Table 2. Formula and chemical composition of weaning phase 2 (4-6 week)

Criteria	Treatment[1]					
	CON	I5	I10	IG2.5	IG5	IG5a
Ingredients, %						
Expanding corn	45.11	45.02	44.93	45.08	44.95	44.99
Soybean meal	17.13	17.15	17.16	17.15	17.16	17.15
Soy-oil	0.62	0.65	0.69	0.65	0.68	0.66
Fish meal	3.50	3.50	3.50	3.50	3.50	3.50
Soytide	6.50	6.50	6.50	6.50	6.50	6.50
Barley	18.00	18.00	18.00	18.00	18.00	18.00
Lactose	7.00	7.00	7.00	7.00	7.00	7.00
L-lysine-HCl, 78%	0.16	0.16	0.16	0.16	0.16	0.16
L-methionine, 99%	0.00	0.00	0.00	0.00	0.00	0.00
L-threonine, 99%	0.01	0.01	0.01	0.01	0.01	0.01
Mono-dicalcium phospate	0.95	0.94	0.93	0.93	0.92	0.92
Limestone	0.79	0.79	0.80	0.80	0.80	0.80
Vitamin Mix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Mineral Mix[3]	0.10	0.10	0.10	0.10	0.10	0.10
ZnO (77.3%)	0.03	0.03	0.03	0.03	0.03	0.008
IMP	0.00	0.05	0.10	0.025	0.05	0.05
GMP	0.00	0.00	0.00	0.025	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Chemical composition[4]						
Maintenance Energy, kcal/kg	3,300	3,300	3,300	3,300	3,300	3,300
Crude protein, %	18.88	18.88	18.88	18.88	18.88	18.88
Lysine[5], %	1.15	1.15	1.15	1.15	1.15	1.15
Methionine[5], %	0.30	0.30	0.30	0.30	0.30	0.30
Threonine[5], %	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
Total Phospate, %	0.60	0.60	0.60	0.60	0.60	0.60

[1] CON: Corn-soybean meal basial diet + ZnO 500 (Phase I) / 300ppm (Phase II); I5: Corn-soybean meal basial diet + IMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); I10: Corn-soybean meal basial diet + IMP 0.10% + ZnO 500 (Phase I) / 300ppm (Phase II); IG2.5: Corn-soybean meal basial diet + IMP 0.025% + GMP 0.025% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5: Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5a: Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm (Phase I / Phase II).

[2] Provided per kg of diet : vitamins per kg of complete diets: vitamin A, 8,000 IU; vivitamin D3,1,800IU;vitaminE,60IU;vitaminK3,2mg;Thiamine,2.00mg;riboflavin,7.0mg;pantothenicacid,25mg;niacin,27mg;pyridoxine,3mg;d-biotin,0.2mg;folicacid,1mg;vitaminB12,0.03mg;

[3] Provided per kg of diet: mineral per kg of complete diet: Se, 0.3 mg; I, 1 mg; Mn, 51.6 mg; Cu, 105 mg; Fe, 150 mg; Zn, 72 mg; Co, 0.5 mg.

[4] Calculated value.

[5] Value means total amino acid (%).

Table 3. Effect of nucleotide on growth performance in weaning pigs:Exp.1

Criteria	Treatment[1]						SEM[2]	P-value
	CON	I5	I10	IG2.5	IG5	IG5a		
Body weight, kg								
Initial	7.49	7.50	7.52	7.50	7.51	7.48	-	0.601
3 week	9.91	9.73	10.07	9.97	9.77	9.60	0.176	0.900
6 week	14.51	15.57	15.68	15.81	15.07	14.17	0.248	0.095
ADG, g								
0-3 week	115	106	122	118	108	101	5.8	0.914
4-6 week	219	278	267	278	252	218	9.6	0.117
0-6 week	167	192	194	198	180	159	5.5	0.099
ADFI, g								
0-3 week	270	238	258	271	246	233	6.5	0.192
4-6 week†, #, T	348^{B}	521^{A}	478^{A}	498^{A}	532^{A}	466^{A}	20.1	0.023
0-6 week	309	380	369	385	388	350	11.4	0.109
G:F ratio								
0-3 week	0.424	0.441	0.464	0.422	0.436	0.432	0.0164	0.987
4-6 week[t]	0.682	0.544	0.559	0.605	0.494	0.467	0.0318	0.348
0-6 week	0.551	0.510	0.525	0.531	0.471	0.456	0.0156	0.387

[1] CON: Corn-soybean meal basial diet + ZnO 500 / 300ppm;
I5: Corn-soybean meal basial diet + IMP 0.05% + ZnO 500 / 300ppm;
I10: Corn-soybean meal basial diet + IMP 0.10% + ZnO 500 / 300ppm;
IG2.5: Corn-soybean meal basial diet + IMP 0.025% + GMP 0.025% + ZnO 500 / 300ppm;
IG5:Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 500 / 300ppm;
IG5a:Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm.

[2] Standard error of the mean.

† Linear response ($P < 0.05$) to different kind of nucleotide amoung CON, IG2.5, IG5, treatments.

Quadratic response ($P < 0.05$) to different kind of nucleotide amoung CON, I5, I10 treatments.

[ABC] Means with different superscripts in the same row significantly differ ($P < 0.05$).

T T-test response ($P < 0.05$) to different level of ZnO through IG5 and IG5a treatments.

[t] T-test response ($P < 0.10$) to different level of ZnO through IG5 and IG5a treatments.

Table 4. Effect of nucleotide on blood profile in weaning pigs:Exp.1

Criteria	Treatment[1]						SEM[2]	P-value
	CON	I5	I10	IG2.5	IG5	IG5a		
IgG, mg/ml								
Initial	1.17							
3 week	2.04	1.73	2.16	2.16	2.73	2.53	0.028	0.165
6 week	3.31	3.06	3.27	3.07	3.77	3.19	0.025	0.786
IgA, mg/m								
Initial	4.60							
3 week	2.53	2.50	2.25	2.50	2.75	2.75	0.123	0.539
6 week	2.75	3.13	2.75	3.25	2.75	3.13	0.095	0.348
IgM, mg/ml								
Initial	0.36							
3 week	0.39	0.47	0.35	0.47	0.44	0.40	0.431	0.656
6 week	0.60	0.67	0.61	0.65	0.59	0.65	0.610	0.558
TNF-α, pg/ml								
Initial	95.95							
3 week	126.59	127.34	128.19	126.50	121.76	121.89	6.2	0.472
6 week	162.53	163.91	168.28	154.45	160.17	121.56	30.3	0.536
IL-6, pg/ml								
Initial	136.11							
3 week	85.38	95.13	97.85	81.92	83.28	93.28	6.164	0.516
6 week	81.92	88.10	104.21	101.85	90.18	129.14	15.478	0.429

[1] CON: Corn-soybean meal basial diet + ZnO 500 / 300ppm;
I5: Corn-soybean meal basial diet + IMP 0.05% + ZnO 500 / 300ppm;
I10: Corn-soybean meal basial diet + IMP 0.10% + ZnO 500 / 300ppm;
IG2.5: Corn-soybean meal basial diet + IMP 0.025% + GMP 0.025% + ZnO 500 / 300ppm;
IG5:Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 500 / 300ppm;
IG5a:Corn-soybean meal basial diet + IMP 0.05% + GMP 0.05% + ZnO 80 ppm.

[2] Standard error of the mean.

Table 5. Effect of nucleotide on incidence of diarrhea in weaning pigs:Exp.1

Criteria	Treatment						SEM[1]	P-value
	CON	I5	I10	IG2.5	IG5	IG5a		
Diarrhea incidence[2]								
0 - 3 week[T]	3.16[B]	2.92[B]	3.16[B]	3.21[B]	3.13[BC]	3.98[A]	0.096	<0.001
4 - 6 week[T]	2.97[B]	2.79[B]	2.94[B]	2.98[B]	2.98[B]	4.56[A]	0.145	<0.001
0 - 6 week[T]	2.92[B]	2.89[B]	3.05[B]	3.09[B]	3.06[B]	4.27[A]	0.118	<0.001

[1] Standard error of the mean.

[2] Diarrhea incidence : 0 (no occurrence) to 8 (diarrhea on all pigs) : Data were measured by average diarrhea incidence during each phases.

[T] T-test response ($P < 0.05$) to different level of ZnO through IG5 and IG5a treatments.

[AB] Means in a same row with different superscript letters significantly differ ($P<0.05$).

Table 6. Formula of weaning phase 1 (0-3 week):Exp.2

Ingredients, %	Treatment				
	I5	I10	IG2.5	IG5	IG5a
Expanding corn	33.700	33.610	33.700	33.610	33.692
SBM	19.000	19.010	19.000	19.010	19.000
Soy-oil	0.100	0.130	0.100	0.130	0.100
Fish meal	4.000	4.000	4.000	4.000	4.000
Soytide	8.500	8.500	8.500	8.500	8.500
Barley	18.000	18.000	18.000	18.000	18.000
Lactose	14.000	14.000	14.000	14.000	14.0000
L · lysine-HCl, 75%	0.270	0.270	0.270	0.270	0.270
L · methionine, 99%	0.020	0.020	0.020	0.020	0.020
L · threonine, 99%	0.070	0.070	0.070	0.070	0.070
MDCP	1.160	1.160	1.160	1.160	1.160
Limestone	0.890	0.900	0.900	0.900	0.900
ZnO	0.050	0.050	0.050	0.050	0.008
IMP	0.050	0.100	0.025	0.050	0.050
GMP	0.000	0.000	0.025	0.050	0.050
Vit. Mix	0.100	0.100	0.100	0.100	0.100
Min. Mix	0.100	0.100	0.100	0.100	0.100
Sum	100.000	100.000	100.000	100.000	100.000
Chemical Composition[3)]					
ME (kcal/kg)	3,300.00	3,300.00	3,300.00	3,300.00	3,300.00
CP (%)	20.56	20.56	20.56	20.56	20.56
Lysine (%)	1.35	1.35	1.35	1.35	1.35
Methionine (%)	0.35	0.35	0.35	0.35	0.35
Threonine (%)	0.86	0.86	0.86	0.86	0.86
Ca (%)	0.80	0.80	0.80	0.80	0.80
Total P (%)	0.65	0.65	0.65	0.65	0.65

[1)] Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D3,1,600IU; vitamin E, 32IU; d-biotin, 64g; riboflavin, 3.2mg; calcium pantothenic acid, 8mg; niacin, 16mg; vitamin B12, 12g; vitaminK, 2.4mg.

[2)] Provided the following per kilogram of diet : Se, 0.1mg; I, 0.3mg; Mn, 24.8mg; CuSO4, 54.1mg; Fe, 127.3mg; Zn, 84.7mg; Co, 0.3mg.

[3)] Calculated values

Table 7. Formula of weaning phase 2 (4-6 week):Exp.2

Ingredients, %	Treatment				
	I5	I10	IG2.5	IG5	IG5a
Expanding corn	45.020	44.930	45.020	44.950	44.990
SBM	17.150	17.160	17.150	17.160	17.150
Soy-oil	0.650	0.690	0.650	0.680	0.660
Fish meal	3.500	3.500	3.500	3.500	3.500
Soytide	6.500	6.500	6.500	6.500	6.500
Barley	18.000	18.000	18.000	18.000	18.000
Lactose	7.000	7.000	7.000	7.000	7.000
L · lysine-HCl, 75%	0.160	0.160	0.160	0.160	0.160
L · methionine, 99%	0.000	0.000	0.000	0.000	0.000
L · threonine, 99%	0.010	0.010	0.010	0.010	0.010
MDCP	0.940	0.930	0.930	0.920	0.920
Limestone	0.790	0.800	0.800	0.800	0.800
ZnO	0.030	0.030	0.030	0.030	0.030
IMP	0.050	0.100	0.025	0.050	0.050
GMP	0.000	0.000	0.025	0.050	0.050
Vit. Mix	0.100	0.100	0.100	0.100	0.100
Min. Mix	0.100	0.100	0.100	0.100	0.100
Sum	100.000	100.000	100.000	100.000	100.000
Chemical Composition[3)]					
ME (kcal/kg)	3,300.00	3,300.00	3,300.00	3,300.00	3,300.00
CP (%)	18.88	18.88	18.88	18.88	18.88
Lysine (%)	1.15	1.15	1.15	1.15	1.15
Methionine (%)	0.32	0.32	0.32	0.32	0.32
Threonine (%)	0.74	0.74	0.74	0.74	0.74
Ca (%)	0.70	0.70	0.70	0.70	0.70
Total P (%)	0.60	0.60	0.60	0.60	0.60

[1)] Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D3,1,600IU; vitamin E, 32IU; d-biotin, 64g; riboflavin, 3.2mg; calcium pantothenic acid, 8mg; niacin, 16mg; vitamin B12, 12g; vitaminK, 2.4mg.

[2)] Provided the following per kilogram of diet : Se, 0.1mg; I, 0.3mg; Mn, 24.8mg; CuSO4, 54.1mg; Fe, 127.3mg; Zn, 84.7mg; Co, 0.3mg.

[3)] Calculated values

Table 8. Effect of nucleotide on intestine weight in weaning pigs:Exp.2

	Treatment[1)]					SEM[2)]
	I5	I10	IG2.5	IG5	IG5a	
Initial body weight, kg						
	8.84	8.84	8.84	8.84	8.84	0.20
d 14 after weaning						
Body weight, kg	11.03	11.14	11.25	10.91	11.27	2.327
Total intestine,g	952	1,113	1,221	1,147	1,169	233.7
Small intestine,g	569	660	695	657	680	151.4
Large intestine,g	358	430	497	459	466	93.7
Spleen,g	24	23	29	30	23	5.5
Liver,g	379	360	432	393	423	77.9
d 15 after weaning						
Body weight, kg	11.18	11.13	10.90	10.95	11.27	2.206
Total intestine,g	1,252	1,285	1,207	1,209	1,370	316.5
Small intestine,g	704	736	683	716	775	154.2
Large intestine,g	507	520	497	464	565	170.1
Spleen,g	41	29	27	29	29	6.8
Liver,g	457	456	445	464	431	86.4
d 42 after weaning						
Body weight, kg	21.30	19.31	23.17	18.42	22.47	3.866
Total intestine,g	1,956	1,825	1,979	1,489	1,790	393.6
Small intestine,g	1,307	962	1,200	834	1,025	309.6
Large intestine,g	598	826	727	617	720	176.7
Spleen,g	55	37	52	37	45	14.0
Liver,g	890	740	969	567	744	218.4

[1)] I5: corn-SBM based diet with ZnO 500 (0-3 wk)/ 300 (4-6 wk) ppm and IMP 0.05%, 2) I10: corn-SBM based diet with ZnO 500 (0-3 wk)/300 (4-6 wk) ppm and IMP 0.10%, 3) IG2.5: corn-SBM based diet with ZnO 500 (0-3 wk)/300 (4-6 wk) ppm and IMP + GMP 0.05%, 4) IG5: corn-SBM based diet with ZnO 500 (0-3 wk)/300 (4-6 wk) ppm and IMP + GMP 0.10%, IG5a) IG2.5: corn-SBM based diet with ZnO 80 (0-6 wk) ppm and IMP + GMP 0.10%

[2)] Standard error of the mean

Table 9. Effect of nucleotide on intestine weight/BW in weaning pigs:Exp.2

	Treatment[1]					SEM[2]	P – value[3]		
	I5	I10	IG2.5	IG5	IG5a		N	C	N×C
d 14 after weaning									
Total intestine,g/kg	86.31	99.97	108.56	105.19	103.79	7.735	0.33	0.78	0.45
Small intestine,g/kg	51.62	59.25	61.81	60.25	60.34	3.611	0.57	0.81	0.55
Large intestine,g/kg	32.52	38.60	44.24	42.10	41.35	4.046	0.12	0.74	0.30
Spleen, g/kg	2.18	2.12	2.61	2.75	2.10	0.272	0.09	0.92	0.77
Liver, g/kg	34.39	32.32	38.40	36.05	37.59	2.198	0.44	0.60	0.86
d 15 after weaning									
Total intestine,g/kg	111.99	115.48	110.80	110.47	121.56	4.151	0.69	0.91	0.92
Small intestine,g/kg	62.97	66.13	62.72	65.39	68.80	2.233	0.80	0.69	0.99
Large intestine,g/kg	45.38	46.72	45.63	42.37	50.16	2.510	0.68	0.90	0.77
Spleen, g/kg	3.67[a]	2.64[b]	2.54[b]	2.71[b]	2.60[b]	0.422	0.04	0.16	0.06
Liver, g/kg	40.94	40.97	40.86	42.37	38.27	1.330	0.98	0.88	0.88
d 42 after weaning									
Total intestine,g/kg	91.83	94.53	85.43	80.87	79.66	5.873	0.56	0.26	0.50
Small intestine,g/kg	61.39	49.82	51.79	45.28	45.63	5.855	0.54	0.09	0.96
Large intestine,g/kg	28.11	42.78	31.39	33.51	32.04	4.933	0.74	0.60	0.16
Spleen, g/kg	2.61	1.93	2.24	2.03	2.03	0.244	0.89	0.11	0.89
Liver, g/kg	41.78	38.32	41.85	30.80	33.11	4.507	0.71	0.05	0.33

[1] I5: corn-SBM based diet with ZnO 500 (0-3 wk)/ 300 (4-6 wk) ppm and IMP 0.05%, 2) I10: corn-SBM based diet with ZnO 500 (0-3 wk)/300 (4-6 wk) ppm and IMP 0.10%, 3) IG2.5: corn-SBM based diet with ZnO 500 (0-3 wk)/300 (4-6 wk) ppm and IMP + GMP 0.05%, 4) IG5: corn-SBM based diet with ZnO 500 (0-3 wk)/300 (4-6 wk) ppm and IMP + GMP 0.10%, IG5a) IG2.5: corn-SBM based diet with ZnO 80 (0-6 wk) ppm and IMP + GMP 0.10%

[2] Standard error of the mean

[3] N: nucleotide; C: content, I5-IG5

[ab] Means in a same row with different superscript significantly different (P<0.05), I5-IG5a

Table 10. Effect of nucleotide on intestine length in weaning pigs:Exp.2

	Treatment[1]					SEM[2]	P − value[3]		
	I5	I10	IG2.5	IG5	IG5a		N	C	N×C
d 14 after weaning									
Duodenum,cm	353	418	381	460	379	77.0	0.46	0.15	0.88
Jejunum, cm	402	303	427	390	421	123.1	0.53	0.44	0.72
Ileum, cm	483	387	423	223	365	135.8	0.17	0.08	0.50
d 15 after weaning									
Duodenum,cm	493	541	423	404	513	114.9	0.20	0.85	0.66
Jejunum, cm	381	326	355	504	274	113.6	0.21	0.42	0.11
Ileum, cm	264	234	390	297	357	103.2	0.11	0.28	0.57
d 42 after weaning									
Duodenum,cm	333[b]	323[b]	471[a]	362[b]	517[a]	64.5	0.03	0.12	0.18
Jejunum, cm	724	607	477	551	482	124.4	0.03	0.73	0.15
Ileum, cm	415	491	549	456	439	89.4	0.36	0.87	0.13

[1] I5: corn−SBM based diet with ZnO 500 (0−3 wk)/ 300 (4−6 wk) ppm and IMP 0.05%, 2) I10: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP 0.10%, 3) IG2.5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.05%, 4) IG5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.10%, IG5a) IG2.5: corn−SBM based diet with ZnO 80 (0−6 wk) ppm and IMP + GMP 0.10%

[2] Standard error of the mean

[3] N: nucleotide; C: content, I5−IG5

[ab] Means in a same row with different superscript significantly different (P<0.05), I5−IG5a

Table 11. Effect of nucleotide on duodenum wall in weaning pigs:Exp.2

	Treatment[1]					SEM[2]	P − value[3]		
	I5	I10	IG2.5	IG5	IG5a		N	C	N×C
d 14 after weaning									
Villus height, μm	267	236	255	246	256	36.9	0.99	0.95	0.90
Crypt depth, μm	278	283	217	210	238	63.3	0.33	0.78	0.45
Villus height: Crypt depth	1.02	0.87	1.20	1.37	1.07	0.386	0.57	0.81	0.55
d 15 after weaning									
Villus height, μm	304^{a}	321^{a}	292^{ab}	337^{a}	241^{b}	43.3	0.88	0.99	0.97
Crypt depth, μm	446^{A}	441^{A}	488^{A}	512^{A}	244^{B}	103.3	0.69	0.91	0.92
Villus height: Crypt depth	0.68^{b}	0.73^{b}	0.60^{b}	0.66^{b}	0.99^{a}	0.163	0.80	0.69	0.99
d 42 after weaning									
Villus height, μm	331	271	279	332	337	64.1	0.84	0.20	0.59
Crypt depth, μm	256	652	367	314	335	253.0	0.56	0.26	0.50
Villus height: Crypt depth	1.32	0.58	0.79	1.16	1.03	0.423	0.54	0.09	0.96

[1] I5: corn−SBM based diet with ZnO 500 (0−3 wk)/ 300 (4−6 wk) ppm and IMP 0.05%, 2) I10: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP 0.10%, 3) IG2.5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.05%, 4) IG5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.10%, IG5a) IG2.5: corn−SBM based diet with ZnO 80 (0−6 wk) ppm and IMP + GMP 0.10%

[2] Standard error of the mean

[3] N: nucleotide; C: content, I5−IG5

[ab] Means in a same row with different superscript significantly different (P<0.05), I5−IG5a

[AB] Means in a same row with different superscript highly significantly different (P<0.01), I5−IG5a

Table 12. Effect of nucleotide on jejunum wall in weaning pigs:Exp.2

	Treatment[1)]					SEM[2)]	P − value[3)]		
	I5	I10	IG2.5	IG5	IG5a		N	C	N×C
d 14 after weaning									
Villus height, μm	241	215	290	383	374	108.7	0.12	0.74	0.30
Crypt depth, μm	221	248	255	229	200	46.4	0.09	0.92	0.77
Villus height: Crypt depth	1.08	0.91	1.14	1.80	1.82	0.552	0.44	0.60	0.86
d 15 after weaning									
Villus height, μm	317	324	328	310	331	28.840	0.68	0.90	0.77
Crypt depth, μm	344	350	389	379	290	51.508	0.04	0.16	0.06
Villus height: Crypt depth	0.93	0.93	0.85	0.81	1.18	0.190	0.98	0.88	0.88
d 42 after weaning									
Villus height, μm	305	274	261	263	280	47.5	0.74	0.60	0.16
Crypt depth, μm	331	279	343	271	306	50.6	0.89	0.11	0.89
Villus height: Crypt depth	0.92	0.99	0.77	0.97	0.91	0.153	0.71	0.05	0.33

[1)] I5: corn−SBM based diet with ZnO 500 (0−3 wk)/ 300 (4−6 wk) ppm and IMP 0.05%, 2) I10: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP 0.10%, 3) IG2.5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.05%, 4) IG5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.10%, IG5a) IG2.5: corn−SBM based diet with ZnO 80 (0−6 wk) ppm and IMP + GMP 0.10%

[2)] Standard error of the mean

[3)] N: nucleotide; C: content, I5−IG5

[ab] Means in a same row with different superscript significantly different (P<0.05), I5−IG5a

Table 13. Effect of nucleotide on ileum wall in weaning pigs:Exp.2

	Treatment[1)]					SEM[2)]	P − value[3)]		
	I5	I10	IG2.5	IG5	IG5a		N	C	N×C
d 14 after weaning									
Villus height, μm	243	331	243	393	260	103.8	0.46	0.15	0.88
Crypt depth, μm	260	296	259	249	236	55.3	0.53	0.44	0.72
Villus height: Crypt depth	0.94	1.20	1.00	1.57	1.13	0.424	0.17	0.08	0.50
d 15 after weaning									
Villus height, μm	290	307	281	283	304	51.1	0.20	0.85	0.66
Crypt depth, μm	338^{A}	349^{A}	375^{A}	375^{A}	213^{B}	68.2	0.21	0.42	0.11
Villus height: Crypt depth	0.87^{b}	0.88^{b}	0.75^{b}	0.75^{b}	1.40^{a}	0.293	0.11	0.28	0.57
d 42 after weaning									
Villus height, μm	311	277	368	261	290	66.1	0.03	0.12	0.18
Crypt depth, μm	387^{A}	231^{C}	342^{AB}	229^{C}	295^{B}	7.0	0.03	0.73	0.15
Villus height: Crypt depth	0.80	1.21	1.07	1.15	0.99	0.209	0.36	0.87	0.13

1) I5: corn−SBM based diet with ZnO 500 (0−3 wk)/ 300 (4−6 wk) ppm and IMP 0.05%, 2) I10: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP 0.10%, 3) IG2.5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.05%, 4) IG5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.10%, IG5a) IG2.5: corn−SBM based diet with ZnO 80 (0−6 wk) ppm and IMP + GMP 0.10%

2) Standard error of the mean

3) N: nucleotide; C: content, I5−IG5

ab Means in a same row with different superscript significantly different (P<0.05), I5−IG5a

ABC Means in a same row with different superscript highly significantly different (P<0.01), I5−IG5a

Table 14. Effect of nucleotide on incidence of diarrhea in weaning pigs:Exp.2

	Treatment[1]					SEM[2]	P − value[3]		
	I5	I10	IG2.5	IG5	IG5a		N	C	N×C
Diarrhea incidence, %									
0−2 week	25.44	25.78	24.56	25.22	24.78	1.851	0.61	0.72	0.91
3−6 week	49.26	46.94	50.83	49.91	46.94	2.850	0.24	0.39	0.71
Diarrhea score									
0−2 week	2.00	2.15	2.00	2.00	2.25	0.240	0.43	0.43	0.43
3−6 week	1.38	1.31	1.23	1.46	1.48	0.232	0.94	0.39	0.13

[1] I5: corn−SBM based diet with ZnO 500 (0−3 wk)/ 300 (4−6 wk) ppm and IMP 0.05%, 2) I10: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP 0.10%, 3) IG2.5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.05%, 4) IG5: corn−SBM based diet with ZnO 500 (0−3 wk)/300 (4−6 wk) ppm and IMP + GMP 0.10%, IG5a) IG2.5: corn−SBM based diet with ZnO 80 (0−6 wk) ppm and IMP + GMP 0.10%

[2] Standard error of the mean

[3] N: nucleotide; C: content, I5−IG5

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Ⅴ. Summary in Korean

본 연구는 자돈 사료 내에서 성장 촉진형 항생제 사용이 금지됨에 따라 성장 성적을 향상시키고 강한 항균 효과를 갖으며 영양소 소화율을 높인다는 공통점이 있는 핵산의 첨가에 따른 자돈에 있어 효과를 평가해보기 위해 이번 실험이 수행 되었다. 본 실험은 2번의 사양실험을 통해 성장성적, 혈액분석, 영양소 소화율, 소장의 형태학적 변화, 설사 빈도를 측정하였다. 사양실험1을 위해 평균체중 7.17 ± 1.176 kg의 삼원교잡종 ([Yorkshire × Landrace] × Duroc) 이유자돈 288두를 공시하여 총 6주 동안 (자돈 전기 3주와 자돈 후기 3주) 실험을 수행하였다. 사양실험2을 위해 평균체중 8.84 ± 0.2 kg의 삼원교잡종 ([Yorkshire × Landrace] × Duroc) 이유자돈 45두를 공시하여 총 6주 동안 (자돈 전기 3주와 자돈 후기 3주) 실험을 수행하였다. 사양실험 1의 실험돈들은 체중과 성별에 따라 6처리 6반복, 펜 당 8마리씩 난괴법 (RCBD; randomized complete block design)으로 배치하여 수행되었고, 사양실험 2의 실험돈들은 체중과 성별에 따라 5처리 1반복, 펜 당 9마리씩 난괴법 (RCBD; randomized complete block design)으로 배치하여 수행되었다. 실험 처리구는 다음과 같다. 실험사료의 경우, 처리구는 다음과 같다: CON: 옥수수-대두박 기초사료 + ZnO 500 (Phase I) / 300ppm (Phase II); I5: 옥수수-대두박 기초사료 + IMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); I10: 옥수수-대두박 기초사료 + IMP 0.10% + ZnO 500 (Phase I) / 300ppm (Phase II); IG2.5: 옥수수-대두박 기초사료 + IMP 0.025% + GMP 0.025% + ZnO 500 (Phase I) / 300ppm

(Phase II); IG5: 옥수수-대두박 기초사료 + IMP 0.05% + GMP 0.05% + ZnO 500 (Phase I) / 300ppm (Phase II); IG5a: 옥수수-대두박 기초사료 + IMP 0.05% + GMP 0.05% + ZnO 80 ppm (Phase I / Phase II).

사양실험 1의 결과, 자돈 전기구간에선 체중 (BW), 일당증체량 (ADG), 일당사료섭취량 (ADFI) 그리고 사료 요구율 (G:F ratio)에서 처리구별 유의적인 차이는 나타나지 않았다. 자돈 후기구간에선 일당사료섭취량 항목에서 처리구별 유의적인 차이가 나타났으며 CON 처리구의 수치가 다른 처리구들에 비해 유의적으로 낮게 나타났다 ($P < 0.05$). 자돈 사료 내 핵산의 첨가는 이유자돈의 성장성적에 있어 긍정적인 영향을 미치는 것으로 사료 되며 핵산의 첨가량을 IMP 0.025% + GMP 0.025% 과 IMP 0.05% 첨가 하였을 때 성장성적을 촉진 한다. 혈액성상의 경우 사료 내 핵산의 첨가를 IMP 0.025% + GMP 0.025% 과 IMP 0.05% 까지 해도 혈액 내 immunoglobulin (IgG, IgA, IgM), TNF-α 그리고 IL-6 과 관련하여 부정적인 영향은 나타나지 않았으며 산화아연의 첨가수준 또한 80 ppm 까지 내려도 혈액성상에 있어 부정적인 영향은 없는 것으로 사료 된다. 실험 전기간 동안 혈액성상, 영양소 소화율, 설사 빈도에 있어서는 통계적인 유의적 차이가 나타나지 않았다.

사양실험 2의 결과, *E.coli* challenge 상황 하에서 종류별 핵산의 첨가 및 첨가량 조절이 소장, 대장 및 간의 무게에는 영향을 미치지 않았지만, 비장의 무게에 있어서는 IMP 단독 사용보다는 IMP와 GMP를 혼합하여 사용하는 것이 소장 발달 및 방어능력 개선에 긍정적인 영향을 미치는 것으로 판단된다. 또한 모든 항목에서 사료 내 ZnO를 감량하여도 다른 처리구와 유의적인 차이가 나타나지 않았다.

이를 통해 사료 내 ZnO를 핵산이 대체할 수 있을 것으로 기대된다. 또한, 소장의 길이에 있어 IMP의 단독 사용보다는 IMP와 GMP를 혼합하여 쓰는 것이 더 나을 것으로 사료되며 ZnO를 대체할 수 있는 것으로 판단된다. 소장 벽의 morphology에 있어 IMP와 GMP의 혼합제재 사용이 공격접종 이후 발생하는 손상을 방지하고 회복을 돕는 것으로 사료되며, 핵산제재 사용이 사료 내 ZnO의 사용을 대체할 수 있을 것으로 사료된다.

결론적으로 IMP 0.05%, IMP 0.025% + GMP 0.025% 첨가는 체중, 일당증체량, 일일사료섭취량, 사료 효율에 있어서 가장 높은 성적을 나타냈다. 또한, 소장의 형태학적 변화에 있어서도 대체로 다른 처리구의 자돈들보다 더 긴 융모 길이와 융와 깊이를 보여 영양소 흡수 및 이용에 있어 가장 긍정적인 영향을 미쳤다.

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