ผลของสารสกัดโปรตีนและแมนแนนโอลิโกแซคคาไรด์จากยีสต์ ต่อสมรรถภาพการเจริญเติบโต สัณฐานวิทยาของลำไส้ และ จำนวนเซลล์ที่สร้าง IgA ของไก่เนื้อที่ได้รับเชื้อซัลโมเนลล่า เอนเทอริกา ซีโรวาร์ เอนเทอริติดิส

นางสาว สวรรยา ศิริบพิตร

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

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EFFECTS OF PROTEIN EXTRACT AND MANNAN-OLIGOSACCHARIDE FROM YEAST SUPPLEMENTATION ON GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY AND ILEAL IgA PRODUCING CELLS OF BROILERS CHALLENGED WITH *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS

Miss Swanya Siribopitr

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Animal Physiology
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Thesis Title	EFFECTS OF PROTEIN EXTRACT AND MANNAN-OLIGOSACCHARIDE FROM YEAST SUPPLEMENTATION ON GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY AND ILEAL IgA PRODUCING CELLS OF BROILERS CHALLENGED WITH *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS
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สวรรยา ศิริบพิตร : ผลของสารสกัดโปรตีนและแมนแนนโอลิโกแซคคาไรด์จากยีสต์ ต่อสมรรถภาพการเจริญเติบโต สัณฐานวิทยาของลำไส้ และ จำนวนเซลล์ที่สร้าง IgA ของไก่เนื้อที่ได้รับเชื้อซัลโมเนลล่า เอนเทอริกา ซีโรวาร์ เอนเทอริติดิส. (EFFECTS OF PROTEIN EXTRACT AND MANNAN-OLIGOSACCHARIDE FROM YEAST SUPPLEMENTATION ON GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY AND ILEAL IgA PRODUCING CELLS OF BROILERS CHALLENGED WITH *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS.) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. น.สพ. ดร. กฤษ อังคนาพร ; อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ส.พญ. อินทิรา กระหม่อมทอง, ผศ. น.สพ. ดร. นพดล พิฬารัตน์, 65 หน้า.

ในการทดลองนี้มีวัตถุประสงค์ในการเปรียบเทียบผลของสารสกัดโปรตีนและแมนแนนโอลิโกแซคคาไรด์ (MOS) จากยีสต์ที่มีต่อสมรรถภาพการเจริญเติบโต, จำนวนเซลล์ที่สร้าง IgA, สัณฐานวิทยาของลำไส้ และการปนเปื้อนของเชื้อซัลโมเนลล่าในไก่เนื้อที่ได้รับเชื้อซัลโมเนลล่า เอนเทอริกา ซีโรวาร์ เอนเทอริติดิส (SE) โดยใช้ไก่เนื้อพันธุ์อาร์เบอร์ เอเคอร์ อายุ 1 วัน จำนวน 575 ตัว แบ่งออกเป็น 5 กลุ่ม (กลุ่มละ 5 ซ้ำ ซ้ำละ 23 ตัว) โดยแบ่งกลุ่มการทดลองดังนี้ T1) กลุ่มที่ได้รับอาหารพื้นฐาน T2) กลุ่มที่ได้รับอาหารพื้นฐานร่วมกับการป้อนเชื้อซัลเนลล่า T3) กลุ่มที่ได้รับอาหารพื้นฐานที่เสริม 2% สารสกัดโปรตีน (ในอาหารไก่ระยะ Starter) ร่วมกับการป้อนเชื้อซัลเนลล่า T4) กลุ่มที่ได้รับอาหารพื้นฐานที่เสริม 2%,1% และ 0.5% MOS (ในอาหารไก่ระยะ Starter, Grower และ Finisher ตามลำดับ) ร่วมกับการป้อนเชื้อซัลเนลล่า และ T5) กลุ่มที่ได้รับอาหารพื้นฐานที่เสริมสารเสริมอาหารทั้ง 2 ชนิดร่วมกับการป้อนเชื้อซัลเนลล่า ทำการตรวจวัดคุณภาพและปริมาณเชื้อซัลโมเนลล่าจากตัวอย่างส่วนตับและม้ามรวมกัน และส่วน ileo-cecal content โดยใช้วิธีการตรวจสอบทางจุลพยาธิวิทยา ทำการวิเคราะห์สัณฐานวิทยาของลำไส้โดยใช้วิธีการตรวจสอบทางจุลพยาธิวิทยา ผลการทดลองพบว่าการเสริมสารสกัดโปรตีนและ MOS ทำให้อัตราการเจริญเติบโตและอัตราการเปลี่ยนอาหารเป็นน้ำหนักดีขึ้นอย่างมีนัยสำคัญในระยะ starter การเสริมสารสกัดโปรตีนและ MOS ช่วยลดปริมาณเชื้อซัลโมเนลล่า ($P<0.05$) ทั้งในส่วนของตับและม้ามรวมกัน และ ileo-cecal content ในไก่อายุ 15 วัน ในวันที่ 22 พบว่าในกลุ่มที่ได้รับสารสกัดโปรตีนมีความสูงของวิลไลในลำไส้ส่วน duodenum สูงกว่ากลุ่มที่ได้รับเชื้อซัลโมเนลล่าเพียงอย่างเดียวในระยะแรก ($P<0.05$) ในระยะท้ายพบว่ากลุ่มที่ได้รับสารสกัดโปรตีนร่วมกับ MOS มีการพัฒนาขึ้นและมีแนวโน้มของสัดส่วน VC ratio สูงกว่ากลุ่มอื่น ในตัวอย่างลำไส้ส่วน jejunum และ ileum ในวันที่ 22 และ 43 พบว่าทุกกลุ่มที่ได้รับสารเสริมอาหารมี VC ratioสูงกว่ากลุ่มที่รับเชื้อซัลโมเนลลาเพียงอย่างเดียวเล็กน้อย ($P>0.05$) การป้อนเชื้อซัลโมเนลลาส่งผลให้จำนวนเซลล์ที่สร้าง IgA ใน ileal mucosa เพิ่มขึ้น การให้สารเสริมทั้ง 2 ชนิดไม่ทำให้เซลล์ที่สร้าง IgA เพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มที่ได้รับเชื้อซัลโมเนลลาเพียงอย่างเดียว การทดลองนี้สรุปได้ว่าสารสกัดโปรตีนและ MOS มีส่วนช่วยลดการติดเชื้อซัลโมเนลล่าในลำไส้และตับม้าม ช่วยพัฒนาลักษณะทางจุลกายวิภาคของเยื่อบุลำไส้เล็กให้ดีขึ้น สารเสริมทั้ง 2 ชนิดไม่มีผลเปลี่ยนแปลงจำนวนเซลล์ที่สร้าง IgA ในลำไส้เล็กส่วนปลาย

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SWANYA SIRIBOPITR : EFFECTS OF PROTEIN EXTRACT AND MANNAN-OLIGOSACCHARIDE FROM YEAST SUPPLEMENTATION ON GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY AND ILEAL IgA PRODUCING CELLS OF BROILERS CHALLENGED WITH *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS. ADVISOR : ASSOC. PROF. KRIS ANGKANAPORN, D.V.M, Ph.D. ; CO-ADVISOR : ASSOC. PROF. INDHIRA KRAMOMTONG, D.V.M., M.S., ASST. PROF. NOPPADON PIRARAT, D.V.M., Ph.D. ; 65 PP.

This experiment aimed to evaluate the effects of protein extract and mannan-oligosaccharide (MOS) from yeast on growth performance, intestinal morphology, Ileal IgA producing cells and Salmonella contamination of broilers challenged with nalidixic acid resistance *Salmonella enterica* serovar Enteritidis (SE). Five hundred and seventy five, 1 day old, male Arbor Acres broiler chicks were allocated into 5 treatments (5 replicates of 23 chicks). The treatments were: T1) Commercial basal diet. T2) Commercial basal diet and challenged with SE. T3) Commercial basal diet supplemented with 2% yeast protein extract (in starter diets) and challenged with SE. T4) Commercial basal diet supplemented with 2%, 1% and 0.5% MOS (in starter, grower and finisher diets, respectively) and challenged with SE. and T5) Commercial basal diet supplemented both additives and challenged with SE. Quantitative and qualitative Salmonella examination in liver-spleen and ileo-cecal content were performed using microbiological assay. Intestinal morphology was analyzed by histopathological assay. The results showed that dietary inclusion of protein extract and MOS significantly improved average daily gain and FCR during the starter period. Dietary inclusion of protein extract and MOS significantly reduced populations of SE in both ileo-caecal content and liver spleen pools at day 15 of age. At day 22, the duodenal villus height of chicks receiving yeast protein was higher than those challenged with SE alone ($P<0.05$). In grower and finisher period, the yeast protein group and MOS group had greater Villus:Crypt (VC) ratio than other groups. For jejunal and ileal morphology at day 22 and 43, all of supplemented groups have VC ratio slightly better than positive control. *Salmonella* inoculation increase IgA producing cells in ileal mucosa and supplementation of both additives had no positive effect compare with positive control. It is concluded that yeast protein extract and MOS helped to ameliorate the adverse effect of SE resulting in the improved intestinal morphology and less contamination of SE in gut and liver spleen pools of chicks. There was no positive effect of both additives on IgA producing cells.

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Field of Study : .Animal physiology	Advisor's Signature........................
Academic Year : 2010	Co-advisor's Signature........................
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LIST OF ABBREVIATIONS

ADG	average daily gain
BPW	buffer peptone water
BW	body weight
CFU	colony forming unit
ADFI	average daily feed intake
FCR	feed conversion ratio
kg	kilogram
mg	milligram
MIL	motility indole lysine medium
min	minute
ml	milliliter
MOS	mannan-oligosaccharide
MSRV	modified semi-rappaport vassiliadis
SE.	*Salmonella* Enteritidis
TSA	tryptic soy agar
TSI	triple sugar iron
XLT_4	xylose lysine tergitol 4
VC ratio	Villus:Crypt ratio
YP	yeast protein extract
μl	microliter
μm	micrometer
μmol	micromole

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CHAPTER I

INTRODUCTION

The most important period for the broiler is the first week after hatching. The high quality food can affect growth in this period. During the first 2 weeks after hatch, chickens have limited capability to produce digestive enzymes since the digestive system is still immature (Leeson and Summer, 2005). Moreover, the transition of dietary sources from protein and fat in egg yolk during embryonic period to complex carbohydrate, protein and fat in conventional feed directly affect gut development. Moreover, chickens are dependent on innate immune from hen in this first period (Cutler, 2002) to prevent infection during immature immune system. In several years, various supplements have been developed to improve performance and feed efficiency extensively. At present, antibiotic has been banned in many countries because of customer insight and concerns of antibiotic resistant pathogenic bacteria as well as antibiotic residues in animal products. Moreover, antibiotic has been severely limited or eliminated in many countries (Eseceli et al.,2010). There are more researches focusing on post-hatch supplement to substitute antibiotic growth promoter. Previous study showed that good pre-starter diet promoted body weight gain.

Protein extract from yeast is abundant in the beneficial substances eg. nucleotides, inositol, glutamate, essential amino acids and peptides, minerals and vitamins. Nucleotides are necessary in improving and developing intestinal integrity and immune response (Grimble and West Wood, 2002). Mannan-oligosaccharide (MOS) from the cell wall of yeast *Saccharomyces cerevisae* has been reported to provide binding sites for bacteria, expectedly reducing pathogen binding to enteric cells. MOS are groups of indigestible oligosaccharides that can bind to pathogenic bacteria and excrete into the faces. In addition, these compounds induce immune response against pathogenic bacteria that usually bound with epithelial host cells (Ferket et al., 2002). Swanson et al. (2002) reported that MOS supplementation in dog increased total lymphocyte count and tended to increase serum IgA concentrations. Previously, a few

studies about effect of immunization and immune system by supplemented yeast protein extract and MOS in broilers challenged with *Salmonella enteric* serovar Enteritidis were demonstrated.

Salmonella is one of the major pathogens in food contaminant affecting human health (Carvajal et al., 2008). The food that usually contaminated is products from chickens and eggs (Rodrigue et al., 1990). During 10-15 years ago, problem of *Salmonella enteritidis* infection have been more increase in poultry industry in US and EU (Holt, 1995). In Thailand Salmonella started to spread around year 1990 and rapidly increasing during the years 1991-1992 (Hampton et al., 1995). The poultry industry has been markedly affected by Salmonella contamination in meat and egg. Thailand is situation the tropical zone that is suitable for bacterial growth, so it is difficult to control the spread of the infection. Antibiotic used to be a major addition to reduce the infection including antibiotic growth promoter (AGP) as preventive measure. However, the ban of AGP due to resistance and contamination is inevitable (Ratcliff, 2002). Many alternatives to replace AGP were used in the poultry industry.

This study aimed to evaluate the effects of protein and mannan-oligosaccharide from yeast on growth performance, intestinal morphology and number of IgA producing cells of broilers challenged with *Salmonella enterica* serovar Enteritidis.

CHAPTER II

LITERATURE REVIEW

2.1 Important of *Salmonella* spp.

2.1.1 The organism

The genus of *Salmonella* is a member of Enterobacteriaceae family. They are rod-shaped, gram-negative, non-spore-forming, predominantly motile enterobacteria with flagella and most isolates exist in two phases: a motile phase I and a nonmotile phase II. They can produce hydrogen sulfide which can be detected by media containing ferrous sulfate, such as TSI (Clark and Barret, 1987). They can grow well under both of aerobic and anaerobic conditions and can survive in variety environment conditions and nutritional substrate. *Salmonella* can grow over a range of about 5° - 45° C, but the optimum temperature is 37°C. The optimum pH is approximately 7.0 but they can grow over range of about 4.0 – 9.0 (Bangtrakulnonth, 2002). The genus of *Salmonella* comprised two species, *S. enterica* and *S. bongori.* The *S. enterica* is divided into six subspecies and *S. enterica* serovar Enteritidis (SE) is one of over 2,500 serovars divided by serology (Popoff and Bockemuhl, 2004). The serotyping of *Salmonella* is a result of antibody interactions with bacterial surface antigens (somatic O antigens, flagella H antigens and V_I). O antigens occur on outer membrane and determined by specific sugar sequences on the cell surface, V_I antigen is a superficial antigen overlying the O antigen and H antigen may occur in changing phase. (Chotikatum, 2005)

2.1.2 *Salmonella* infection

A *Salmonella* infection is a foodborne disease that most infected from eggs and chicken products. The major cause of gastrointestinal disease in humans and animal over the world is *Salmonella enterica* serovar Enteritidis (Poppe, 2000). Infection of

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Salmonella in chickens may be from fed with contaminated feed, water and others in hatchery. The hens that are infected with *Salmonella* can transfer agent to the eggs via ovary, and pass through to cloaca (Nakumura et al., 1993). *Salmonella* colonized and proliferated in the cloaca, which resulted to increasing the number of eggs contaminated. Period after hatching is critical because of chickens have limited microflora in the intestine and are more sensitive to *Salmonella* than the older. In addition, during the first 2 weeks after hatch, chickens also have limited capability to produce digestive enzymes because the digestive system is still immature (Leeson and Summer, 2005). Moreover, the transition of dietary sources from protein and fat in egg yolk during embryonic period to complex carbohydrate, protein and fat in conventional feed directly affect gut development.

2.1.3 Pathogenesis of *Salmonella* infection

The pathogenesis of SE in the chicken started with colonization in the digestive tract. The main area for colonization is the cecum and crop, the pathogen are able to enter via epithelium of intestine. After SE is ingested into the crop, the pH of crop (4-5) is able to prevent some of SE growth but others that tolerate will increase resistance to acidity and pass through the gizzard and proventriculus (Chotikatum, 2005). The proventriculus secreted enzyme for digestion including pepsin and hydrochloric acid. The enzymes were secreted to the gizzard for mixing feed and make the pH of gizzard to approximately 2 to 3 (Heres et al., 2003). Although, the gizzard was high acidity but some of SE can survive. The surviving SE through to the intestinal tract can cross the intestinal epithelium after attachment to receptor substance in mucus and this is considered as the first step in colonization of bacteria within the intestinal mucosa. Penetration of the intestinal mucosa is a summary for the pathogenesis of systemic infection; this process can induce to diarrhea. Invasion occurs specifically via the apical surface, where within a critical distance from cells, SE induces destruction and elongation of microvilli, which precedes endocytosis. Invasion is believed to occur by receptor mediated endocytosis, though receptors have not been identified. The ceca are the site within the intestinal tract of infected chickens that most commonly contain SE

(Fanelli et al., 1988). The ceca were a target organ for SE to amplification and it poses a potential source for environmental contamination (Dhillon et al., 2001). Bacteria are the predominant microbes, specifically obligate anaerobes which are found in the lumen. This has been ascribed to the physical attachment of SE to the cecal epithelium (Soerjadi et al., 1982). This adhesion is most observed in epithelium via the cecal lumen (Desmidt et al., 1998), and it is likely that the uptake of bacteria occurs in mature enterocytes at the villus tips (Amin et al., 1991). After that they penetrate the lamina propria where they replicate, or proceed to deeper tissues. van Immerseel et.al. (2002) indicated that SE were first detected 3 hours post-infection in the caecal lumen and 9 hours post-infection in the cecal lamina propria, SE colonized in Peyer's Patches and invade to M cell. M cell reside throughout the gut as follicular-associated epithelium that overlays lymphoid follicle, e.g., Payer's patches. M cell have been considered to be the most effective cells for the transport of specific antigens from the intestinal lumen into the underlying lymphoid tissue (Charles et al.,1992). The pathogens from tissue infected are shifted to the regional lymph nodes, where macrophages in the lymphatic sinuses were the first effective barrier to prevent the spread. If this host defense mechanism successfully halts bacterial expansion, the infection remains localized to the intestine and the gut- associated lymphoid tissues (GALT). However, if the macrophages are unable to limit spread, SE can cause a systemic disease. The pathogens spread from the GALT via the afferent lymphatic and the thoracic duct into the vena cava, resulting in the systemic infection the capillary systems of liver and spleen constitute an efficient filtering system, which focuses infection to liver and spleen. These organs are usually enlarged during systemic infection (Andreas et al., 2000). SE colonizes in both spleen and liver after an infection of 1 day old chicken. The frequently organs found more positive in the birds are not only the spleen, liver and kidneys but also the less frequent from lungs and heart (Brown et al., 1975). Three general categories of toxin have been reported to play role in the pathogenicity of paratyphoid SE (Gast, 2000). Endotoxin is associated with the lipid portion of SE cell wall lipopolysaccharide (LPS). It will be released into the bloodstream of an infected animal when bacterial cells are lysed, and producing fever (Gast, 2000). LPS also attributes to the resistance of the bacterial cell wall to attack and digestion by host phagocytes. Loss of the ability to synthesize

complete LPS has been impaired ability to colonize the ceca and invade to the spleen in broiler chicks (Craven, 1994). In the same way, SE endotoxin administered intravenously produced liver and spleen lesion in 2 week old (Turnbull and Snoeyenbos, 1973). However, enterotoxin activity by *Salmonella* induces secretory response of epithelial cells that results in fluid accumulation in the intestinal lumen (Koupal et al, 1975). A heat labile enterotoxin of *Salmonella* cause structural damage to intestinal epithelial cells, perhaps by inhibiting protein synthesis (Koo et al., 1984).

2.1.4 The effects of *Salmonella* infection on immunity

The host immune response to pathogens in the earliest stages of infection is a critical determinant of disease resistance and susceptibility. These early responses are dedicated to the containment of the pathogens, holding infections to a level that can be resolved by the ensuing development of acquired immune mechanisms. The innate immune system is a rapidly induced, phylogenetically conserved response of all multicellular organisms (Medzhitov et al., 1997). The protective effect is usually termed competitive exclusion (CE), a process by which an organism is prevented from colonizing a given environment because of the prior presence of other organisms that are better able to establish and maintain themselves in that environment (Pasteur et al., 1878). Although protection of chicks is mainly due to bacterial competition (Weinack et al., 1984), CE treatment may also play an important role in mucosal immunity stimulation, which could contribute significantly to host resistance.

The local immune response in the GI tract has been scarcely studied in relation to CE treatment and is likely to involve a variety of factors, including the role of native microflora, host M cells, and secretory IgA (sIgA). Probiotics, defined as live cultures of microorganisms administered orally, act beneficially on host health; inhibit pathogens; enhance intestinal immunity; and have a protective effect on the gut microflora (Fuller, 1989). There is strong scientific evidence supporting that certain components of the gut microflora are involved in protection of the host against infectious diseases (Wilson and Freter, 1986). Factors affecting intestinal colonization of poultry by SE, and considerations are given to possible means of increasing host resistance to infection.

2.2 Methods in controlling *Salmonella* infection

Antibiotics have been used for many years as growth promoting agents for the chicken industry. The antibiotic growth promoters are modifying the intestinal microflora, especially targeting gram positive bacteria, which are associated poor health and performance of the animals (Bedford, 2000). Antibiotics are commonly used in livestock industry production as dietary additives are bacitracins, lincomycin, penicillin, streptomycin, tetracycline, tiamulin, tylosin and virginiamycin. However, the use of antibiotics for feed additives has lead to drug resistant bacteria and the possible presence of antibiotic residues in poultry products. Then, the resistant bacteria may be remaining in the poultry products, resulting to the people eating it may become sick from the bacteria resistance. Recently, the antibiotics as growth promoters will be banned from use in the EU in 2006 (David, 2006). The antibiotic free chicken for consumer is enormous concern in poultry production.

Most antibiotic growth promoters (AGP) were suspended for almost a decade ago especially in Europe. Many researchers are examined in finding appropriate alternative substances to reduce infection and enhance immunity of the bird e.g. probiotic, prebiotic, yeast protein extract, MOS, FOS and Organic acids etc.

2.2.1 Probiotic

Probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). The most commonly used strains are members of the heterogeneous group of lactic acid bacteria; such as *Lactobacilli*; *Enterococci* and *Bifidobacteria*. There have been numerous studies on probiotics in human and animal nutrition. Mechanisms of probiotic response involve the production of directly inhibitory compounds, reduction of luminal pH through short chain fatty acids production, competition for adhesion site on the gut wall and nutrients and modulation of immune response (Gibson and Fuller,2000 ; Rolfe,2000).

However, selection criteria for probiotic much debate and should be taken into account when defining appropriate strains. The survival of the probiotic was

compromised in the supplement product before ingestion, and even more so in the host after ingestion. The bacteria are encountered by many physiochemical effects i.g. gastric acid and secretions of the small intestine such as bile salts and pancreatic enzymes. Moreover, in the large intestine, the bacteria must compete effectively with a complex and metabolically active indigenous flora (Collins et al., 1999).

2.2.2 Prebiotic

Prebiotics are non-digestible food ingredients that beneficially affected the host by selectively stimulating the growth or activity of bacteria in digestive system and improve host health (Gibson and Roberfroid, 1995). Typically, prebiotics are carbohydrates (such as oligosaccharides), but the meaning may include non-carbohydrates. For a food ingredient to be classified as a prebiotic, it must 1) not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract; 2) be a selective substrate for one or a limited number of potentially or beneficial commensal bacteria in the colon, either stimulating the bacteria to grow, become metabolically activated, or both; and 3) be able as a consequence to alter the colonic microflora toward a more healthier composition (Gibson and Roberfroid, 1999). Thus, survival of foodborne pathogens may be reduced by prebiotics.

2.3 Yeast protein extract

Yeast protein extract (YP) is the common name for various forms of processed yeast products made by extracting the cell contents (removing the cell walls). Yeast extract have Monosodium glutamate (MSG), contains free glutamic acid that created savory flavors and umami taste sensations. Moreover, YP are consists of nucleotide, inositol, protein, vitamins and minerals. Rutz et al. (2004) reported that supplementation with YP on starter period resulted in higher feed intake but only significant up to 14 days of age. The dietary inclusion of YP may benefit early enteric development of young poultry, which may help to resist enteric disease challenges.

2.3.1 Glutamic acid

Figure 2.1 Molecular structure of the glutamic acid (NEUROtiker, 2007)

Glutamate is a key molecule in cellular metabolism. In humans, dietary proteins are broken down by digestion into amino acids, which serve as metabolic fuel for other functional roles in the body. The physiological importance of the amino acid L-glutamine for promoting and maintaining cell function is now widely accepted. The importance of glutamine to cell survival and proliferation in vitro was first reported by Ehrensvard et al. in 1949. Glutamine is quantitatively the most important fuel for intestinal tissue. It is metabolized to glutamate by phosphate-dependent glutaminase. Glutamate undergoes transamination with pyruvate generating L-alanine and 2-oxoglutarate. The latter metabolite is then oxidized in the tricarboxylic acid (TCA) cycle generating malate, which, by the action of NADP+-dependent malic enzyme, generates pyruvate. The NADH and FADH2 generated via this pathway are used for electron donation to the electron transporting chain in the mitochondria and thus promote ATP synthesis. The L-alanine produced in this pathway is exported to the hepatic portal vein for transport to the liver (Kimura et al., 1988). Glutamine is recognized as an important dietary component for the maintenance of gut integrity (Neu et al., 2002) and reduces the degree of derangement induced by mechanical intestinal obstruction (Chang et al., 2001). As a result, glutamine administration reduces bacterial translocation (Erbil, 1999), being beneficial to critically ill and other patients (Newsholme et al., 1987; Boelens et al., 2001). In fact, glutamine has been shown to improve various aspects of medical nutritional care of patients with gastrointestinal disease or cancer, burn victims, postsurgical patients, and low birth weight neonates (Savy, 2002; Gismondo et al., 1998; Neu, 2001). This amino acid also normalizes the AIDS-associated increased intestinal permeability (Thomson et al., 2001).

2.3.2 Nucleotide

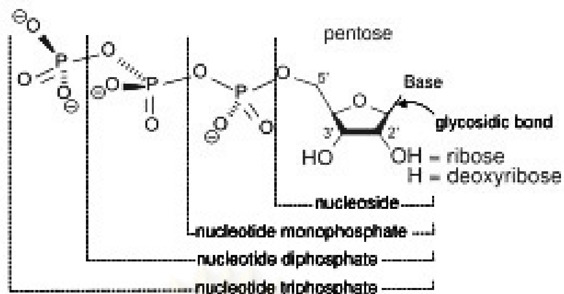

Figure 2.2 Molecular structure of the nucleotide (Boris and Sjef, 2005)

Nucleotides are precursors of DNA and RNA, energy metabolites, secondary messengers that mediate hormones responsible for growth, and the biosynthesis of protein inhibitors that have antibiotic-like properties. A nucleotide is the monomer structural unit of nucleotide chains that form the nucleic acids RNA and DNA, as well as several lesser nucleic acids. The nucleotide consists of a heterocyclic nucleobase, a pentose sugar like ribose or deoxyribose, and a phosphate or polyphosphate group. Nucleotides are normal components of the diet and the body provides mechanisms for their absorption and incorporation into tissues (Sanchez and Gil, 2002). However, during periods of intensive development, nucleotide availability could limit the maturation of fast dividing tissues with low biosynthetic capacity, such as the intestine (Leleiko et al, 1983; Van Buren and Rudolph, 1997). Nucleotides are essential nutrients involved in gut development and repair, skeletal muscle development, heart function and immune response (Grimble and Westwood, 2002) In chickens, dramatic changes occur in the development of the small intestinal mucosa after hatching, including enterocyte maturation, intensive cryptogenesis and villous growth (Geyra et al, 2001). This intestinal development influences the growth rate, since intestinal maturation plays a rate determining role in providing the substrates for growth (Smith et al, 1990). The beneficial effect of nucleotides on intestinal cell integrity, development, and turnover, with significant proliferation of crypt cell was already demonstrated (Tsujinaka et al., 1993; Dell'Orto et al., 2002). Nucleotides also increase intestinal villi length, and improve the immune response of broilers, promoting nutrient absorption and increased weight gain

(Yu et al., 2002). They have also significant effects on enterocytes during intestinal development, maturation, and repair after damage caused by stress or pathogens (McCauley et al., 1998).

2.3.3 Inositol

Figure 2.3 Molecular structure of the inositol (Yikrazuul, 2010)

Insitol is the common name of myoinositol, the only form of inositol utilized by the body as a nutrient. Inositol is a type of sugar related to glucose. It occurs naturally in the body and is present in many foods. Chemical formula of inositol is $C_6O_6H_{12}$. Originally identified in association with the B vitamins, inositol is an essential eukaryotic metabolite that is a component of a major class of membrane phospholipids, and also functions in signal transduction and in a variety of other capacities. One form of inositol, inositol hexaniacinate, has been used to support circulatory health because it functions like niacin in the body. Inositol hexaphosphate, also known as phytate, is conjunction with plant fiber, and is a source of myo-inositol in the diet. The major dietary forms of myo-inositol are inositol hexaphosphate or phytic acid, which is widely found in cereals and legumes and associated with dietary fiber, and myo-inositol-containing phospholipids from animal and plant sources (Clements, 1980). The functions of inositides are as varied as their structures. The additions contains inositol may be enhancement metabolism and health.

2.4 Mannan-oligosaccharide

Mannan-oligosaccharide (MOS) are extracts from the cell wall of yeasts. Mannose is a monosaccharide that forming of MOS. The small intestine has no enzyme to break down MOS bonds; therefore they pass through the small intestine harmless to

the large intestine after ingestion (Strickling et al., 2000). Mannose-based oligosaccharides occur naturally in cells walls of the yeast *Saccharomyces cerevisiae* from yeast culture (Spring et al., 2000). The commercially available product, Bio-Mos (Alltech USA), is a source of MOS. Bio-Mos has shown in suppressing enteric pathogens, modulating the immune response, improving the integrity of the intestinal mucosa, and promoting improved growth and feed conversion in studies with chickens (Spring et al., 2000; Savage and Zakrzewska, 1997). Mannan oligosaccharides improved performance in nursery pigs and weight gain and feed intake in dairy calves (Dvorak and Jacques, 1997). MOS are the primary antigenic components because gram-negative bacteria can attach to the intestinal epithelium using mannose-specific fimbriae (Ofek et al., 1977), MOS provides competitive binding sites for these intestinal pathogens. Multiple strains of *Escherichia coli* and *Salmonella* agglutinated MOS in vitro (Spring et al., 2000). MOS is not enzymatically digested in the small intestine; therefore, bacteria bound to MOS were exit from the intestine without attaching to the epithelium (Spring et al., 2000). Mannan oligosaccharides may also enhance health by stimulating antibody production (Savage et al., 1996) or by affecting intestinal morphology and function (Iji et al., 2001). Inhibition of the bacteria responsible for toxin production could prevent or decrease the severity of diarrhea (Giannella, 1983).

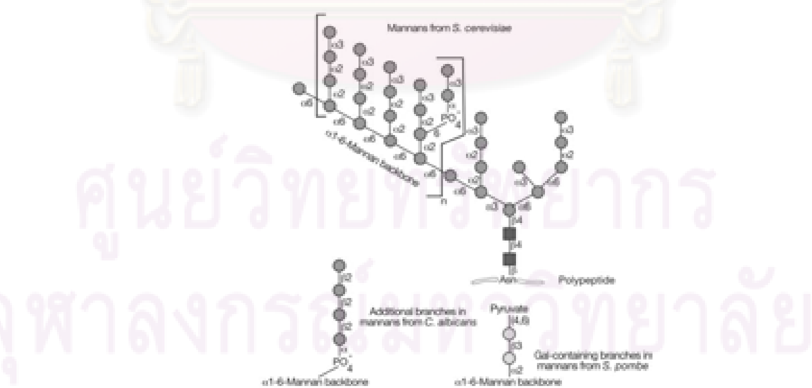

Figure 2.4 Molecular structure of the mannan-oligosaccharide (Yalcinkaya et al., 2008)

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CHAPTER III
MATERIALS AND METHODS

This study was approved by Animal Care and Use Committee of the Faculty of Veterinary Science. Chulalongkorn University, No. 0931023

3.1 Animals and management

Five hundred and seventy five, 1 day old, male Arbor Acres broilers were obtained from *Salmonella enterica* serovar Enteritidis (SE) free farm. Before the experiment started, cloacal swabs were randomly performed on seventy chicks (one sample/chick) examined for Salmonella Spp. Chicks were divided into 5 treatments (5 replicates of 23 chicks) with similar average body weight in each treatment. Chicks were inoculated with 0.3 ml SE culture (10^8 cfu/ml) at day 1 and 1 ml (10^8 cfu/ml) at day 7 post-hatching. All chicks were vaccinated with Newcastle and Infectious Bronchitis diseases at the first day from the hatchery. The control group was raised in a room separated from other SE inoculated groups. The experiment was set in a closed concrete floor house in evaporative cooling facility. Lighting was turned on continuously throughout the experiment. The total experimental period was 42 days.

3.2 Feed and feeding

3.2.1 Diet

Corn-soybean meal basal diets were divided into 3 periods: starter (1-21 days), grower (22-35 days) and finisher (36-42 days). Ingredients and composition of the experimental diet are shown in Table 3.1. Birds were received feed and water *ad libitum* throughout the period of 6 weeks. The diets were 1) basal diet, 2) basal diet supplemented with 2% yeast protein extract (NuPro™, Alltech Inc., USA) in starter period 1-7 days, 3) commercial basal diet supplemented with 2% Mannan-oligosaccharide (MOS™, Alltech Inc., USA) in starter period (7-21 days), 1% in grower period (22-35 days) and 0.5% in finisher period (36-42 days). Chicks in control and SE inoculated group were received diet 1. Chicks in group 3 and group 4 received diets 2 and 3 respectively while chicks in group 5 received both diets 2 and 3 in the respective period.

Table 3.1 Ingredients and composition of the experimental diet.

Ingredients	Amount (kg/100 kg diet)		
	Starter	Grower	Finisher
GR.Corn-S	58.38	58.88	63.89
Lard	1.52	3.40	3.51
Soybean ML.-Hipro	35.07	33.60	28.74
Calcium Carbonate.	1.59	1.30	1.25
MDCP-21	1.95	1.72	1.64
Salt	0.12	0.16	0.16
Sodium Bicarbonate.	0.37	0.31	0.30
DL.Methionine	0.34	0.24	0.20
L-Lysine HCL.	0.27	0.09	0.07
Threonine	0.08	-	-
Choline Chloride (60%)	0.09	0.09	0.08
Antixidant	0.01	0.01	0.01
Anticoccidia	0.05	0.05	
Vitamin premix	0.08	0.05	0.05
Mineral premix	0.10	0.10	0.10
Nutrients			
Crude Protein %	22.00	21.00	19.00
ME Poultry KCal/Kg	3025.00	3150.00	3200.00
Moisture %	11.10	11.00	11.09
Dry Matter %	88.90	89.00	88.91
Fat %	4.10	5.97	6.20
Crude Fibre %	2.83	2.79	2.75
Ash %	6.02	5.47	5.12
Calcium %	1.05	0.90	0.85
Total Phos %	0.80	0.74	0.70
Available Phos-P %	0.50	0.45	0.43
Sodium %	0.16	0.16	0.16
Chloride %	0.23	0.23	0.23
Salt %	0.11	0.15	0.16
Linoleic Acid %	1.68	2.02	2.08
Lysine %	1.43	1.24	1.09
AV Lys_P %	1.31	1.13	0.99
Methionine %	0.67	0.56	0.50
AV Met_P %	0.64	0.54	0.47
Methionine & Cystein %	1.07	0.95	0.86
AV M&C_P %	0.99	0.88	0.79
Threonine %	0.94	0.84	0.76
AV Thr_P %	0.84	0.74	0.68
Tryptophan %	0.27	0.27	0.24
AV Trp_P %	0.25	0.24	0.21
Isoleucine %	1.05	1.02	0.92
AV Ile_P %	0.97	0.94	0.85
Potassium %	0.96	0.93	0.84
Choline mg/Kg	1714.02	1674.71	1512.49
Arginine %	1.51	1.46	1.31
Bulk Density gm/l	726.84	731.44	737.21

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3.2.2 Experimental groups

Salmonella enterica serovar Enteritidis (SE, nal^r) (*Salmonella enterica* serovar Enteritidis, nalidixic acid resistant strain) was obtained from Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University. The SE was kept in stock agar at room temperature cultured to TSA (Tryptic Soy Agar); incubated at 37°C for 24 h and subcultured 2-3 times for pure culture. Chicks in groups 2, 3, 4 and 5 were inoculated with 0.3 ml (10^8 cfu/ml) SE alful at day 1 and 1 ml (10^8 cfu/ml) at day 7 of the experiment. The treatment group is shown in Table 3.2

Table 3.2 The experimental groups

Treatment	Group 1	Group 2	Group 3	Group 4	Group 5
Yeast protein extract (NuPro, Alltech USA)	-	-	✓	-	✓
Mannan-oligosaccharide (Bio-MOS, Alltech USA)	-	-	-	✓	✓
Salmonella enterica serovar Enteritidis inoculation	-	✓	✓	✓	✓

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3.3 Experimental procedure

3.3.1 Protocol of experiment

Experiment period (day)

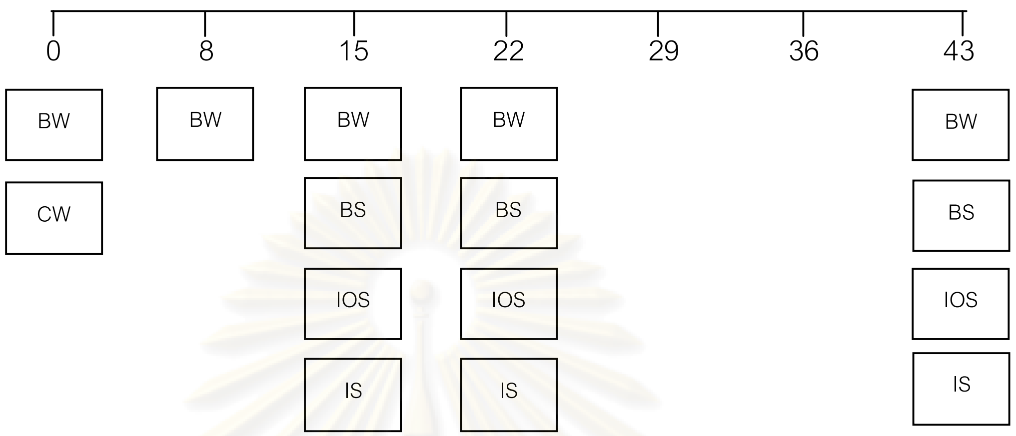

Figure 3.1 Diagram showing the whole period of experiment.

BW = Body weight

BS = Blood sample

CW = Cloacal swabs

IOS = Internal organ sample (liver, spleen)

IS = Intestinal sample (duodenum, jejunum, ileum and cecum)

Weighting feed every sampling time.

3.3.2 Sample collection and tissue preparation

At day 1, cloacal swab using sterile cotton was randomly performed onto buffer peptone water (BPW) in 70 chicks (one sample/chick). Chicks were inoculated with 0.3 ml SE culture (10^8 cfu/ml) and 1 ml at day 7 of the experiment. The chicks were weighed and feed intake recorded at days 1, 21, 35 and 42. Mortality was record daily. Three chicks from each replication were randomly selected and sacrificed with intracardiac injection of pentobarbital sodium (120 mg/kg BW) using 21G, 2.5 inch needle at days 14, 21 and 42. Abdomen was exposed and parts of intestines from duodenum to ileocecal junction were excised and separated. The intestinal section from

the end of pylorus to the entry of bile and pancreatic ducts was taken as duodenum part (D), the section from the entry of bile and pancreatic ducts to a section of Mechel's diverticulum was taken as the jejunal part (J), and the ileal (I) part was taken from Mechel's diverticulum to the ileocecal junction. Liver and spleen pool and the content in ileal and cecal pool were collected and kept at -20°C until analysis for bacteriological examination. Parts of intestine from duodenum to ileum were collected, rinsed with normal saline (NSS) and collected into 10% formalin. The sample were kept at -20°C until analysis for histomorphological examination.

3.3.3 Bacteriological examination

SE culture was kept in stock agar before use. The culture were transferred and streaked on Tryptic Soy Agar (TSA) plate and incubated at 37°C for 18-20 h for purity examination.

3.3.4 Qualitative and Semi-quantitative examination of SE

3.3.4.1 Semiquantitative examination

Approximately 1g of liver and spleen samples were chopped and pooled together, 1g of ileal content, each was put into BPW. From the initial 10^{-1} dilution, 10-fold serial dilutions were made in BPW. The 0.1 ml of BPW was dropped on to MSRV (Modified Semi-Rappaport Vassiliadis) agar plate plus 10µg/ml Novobiocin (NO), 5 drops on plate and incubated at 42°C for 24-48 h. The outermost of colonies from MSRV were transferred and streaked onto XLT_4 (Xylose Lactose Tergitol^{TM} 4) agar plates plus 50µg/ml Nalidixic acid (NA) and incubated at 37°C for 24 h. The pink colonies with black spot were suspected of SE Then, they were further subjected to confirmation test. The SE count was expressed in $\log_{10}$ SE count.

3.3.4.2 Qualitative examination

The samples of cloacal swabs, the samples of liver and spleen and the samples of ileocecal content, 1g weight of each were put into BPW and incubated at 37°C for 24 h. The 10-fold serial dilutions were prepared as similar as semiquantitative examination and dropped on to MSRV following semiquantitative examination. Percentage of positive SE sample found was expressed and analyzed.

3.3.4.3 Confirmation test

The suspected colonies were tested for biochemical assays including glucose fermentation, hydrogen sulfide gas production from TSI (Triple Sugar Iron) agar, motility test, lysine carboxylation and indole production were detected from MIL (Motility Indole Lysine medium). Serogroup of SE (Group D) was confirmed by a slide agglutination test using antiserum of *Salmonella* O polyvalent groups combined, and specific serogroup D.

3.4 Histomorphology and Immunohistochemistry study

3.4.1 Tissue preparation

The tissue samples collected from each part of intestine (duodenum, jejunum and ileum) were kept in 10% formalin and selected 6 sections per treatment in each part. The samples were dehydrated in a series of ascending grades of alcohol (70%, 80%, 95% and 100%), cleared in several times of xylene, and infiltrated with melted paraffin in the oven. The tissues were then embedded in paraffin and the sections were cut at 4 μm thickness using sliding microtome (IVS-410, SAKURA Tissue-Tek, Tokyo, Japan). The sections were floated on warm water in a floatation bath at 37°C for stretching then the sections were put on cleaned glass slides and dried at 37°C in incubator. The sections were deparaffinized first in several times of xylene followed by rehydration in a series of descending grades of alcohol (100%, 95%, 80% and 70%).

3.4.2 Hematoxylin-eosin Staining

The histological sections were stained using Hematoxylin-eosin (Hussun et al., 2009). The sections were hydrated with xylene and differentiate alcohol, after running water stained in hematoxylin 1 min and washed in running water, stained in eosin 2 min and dehydrated with differentiate alcohol and xylene, respectively. Then Villus height and crypt dept were measured from duodenum, jejunum and ileum sections under microscope at 4X. The villus height was measured from the top of the villus to the villus-crypt junction and crypt depth was measured from the villus-crypt junction to the base of the crypt. Ten villi measurements were taken per section (6 sections on slide/treatment) from each part of intestine. Villus/Crypt ratio was calculated.

3.4.3 Immunohistochemical Staining for IgA

Deparaffined slide sections of ileum were pretreated by citrate buffer, pH 6, for 10 min in microwave, washed in PBS and blocked endogenous peroxidase by 3% H_2O_2 for 20 min. Blocking serum by 2% rabbit serum was performed (diluted with 0.01M PBS) for 30 min at 37°C, followed by incubation with goat anti-chicken IgA (1:500) (Bethyl Lab. Inc. USA) for overnight at 4°C. After brief washing with PBS, sections were treated with 1% peroxidase-conjugated rabbit anti-goat IgG (1:100) (Bethyl Lab. Inc. USA) 1h at room temperature. The positive reactions of IgA were exposed by treating with 0.2 mg 3,3'-diaminobenzidine (DAB) pH 7.6 containing 0.03% H_2O_2 and then counterstained few dips with hematoxylin. The IgA positive cells in lamina propria were counted under microscope observation at 4X and choose 4 positions per section (6 sections on slide/treatment) from ileum sections. The IgA positive cells in mucosal line were counted in 4 high power fields of each section (Kamal et al., 2011) and grading: 1= 1-30, 2 = 31-60, 3 = 61-90, 4 = 91-120 and 5 = 121-150 cells/ high power field.

3.5 Calculation of the growth performance

The chickens were weighed at 0, 21, 35 and 42 days old. The feed intake was recorded during days 0-21, 22-35 and 36-42. Number and body weight of dead

chickens were recorded for calculation of mortality and feed conversion ratio (FCR), respectively.

Body weight gain (g/b) = Final body weight – Initial body weight

Average daily gain (ADG, g/b/d) = Body weight gain / Days

Feed intake (g/b) = Total feed intake / Final chick numbers

Average Daily feed intake (ADFI, g/b/d) = Feed intake / Days

Mortality rate (%) = (No. of dead chicks / Total chick numbers) x 100

$$\text{Feed conversion ratio (FCR, kg feeding/kg body weight gain)} = \frac{\text{Total pen feed}}{\text{Final body weight} - \text{Initial body weight}}$$

3.6 Statistical analysis

All data are presented as Mean ± SE. The effects of treatments were analyzed using One-Way Analysis of Variance (ANOVA) under completely randomized design. If the probability of F test was less than 0.10, Duncan's New Multiple Range test was used to compare pair wise means at P<0.05 using SAS program. The qualitative examination used of SE was analyzed by Chi-square test (SAS Institute, 2003).

CHAPTER IV

RESULTS

4.1 Effects of treatments on growth performance

After inoculation of SE (days 1 and 7), It is shown that there were no significant difference in performance during starter period (day 1-21). However, chicks in T5 (YP+MOS) had higher ($P<0.05$) average daily gain (ADG) and slightly better average daily feed intake (ADFI) than other groups. Consequently, this helped to significantly improve feed conversion ratio (FCR) ($P < 0.05$) in T5 group compared to others. There were no significant difference in initial weight and percentage of mortality. (Table 4.1)

Growth performance of chicks in grower-finisher period (day 22-42) is shown in Table 4.2. There were no significant differences in initial weight, ADG, ADFI, FCR and percentage of mortality.

The overall growth performance (day 1-42) is shown in Table 4.3. Likewise the grower-finisher period, there were no significant differences in all data except for ADFI. The final body weights of chicks in all groups were low compared to the breed specification at day 42. Chicks in all additives supplemented groups had significantly better ADFI ($P < 0.05$) than chicks that were inoculated with SE alone. In addition chicks that received both supplements (T5) tended to have the best performance ($P > 0.05$) when considered on final body weight, ADG and FCR. Percentages of mortality were only found during starter period and no significant difference was demonstrated among group of chicks.

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Table 4.1 Effect of treatments on growth performance of broiler chickens during starter period (days 1-21)

	Treatment					
	T1(CON)	T2 (SE)	T3(SE+YP)	T4(SE+MOS)	T5(SE+YP+MOS)	P-value
Initial weight (g/b)	43.8 ± 0.32	43.4 ± 0.09	43.5 ± 0.00	43.6 ± 0.09	43.6 ± 0.09	0.41
ADG (g/b/d)	27.91 ± 0.30^{b}	27.85 ± 0.24^{b}	27.92 ± 0.27^{b}	28.32 ± 0.14^{ab}	28.75 ± 0.27^{a}	0.09
ADFI (g/b/d)	37.30 ± 0.18	37.28 ± 0.19	37.54 ± 0.14	37.47 ± 0.10	37.58 ± 0.10	0.47
FCR	1.34 ± 0.01^{a}	1.34 ± 0.01^{a}	1.35 ± 0.02^{a}	1.33 ± 0.01^{a}	1.29 ± 0.01^{b}	0.02
Mortality (%)	0.00	0.00	0.00	0.87 ± 0.87	1.74 ± 1.07	0.21

Mean ± S.E., n = 5

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide

$^{a, b}$ Means in the same row with unlike superscripts differed significantly ($p<0.05$)

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Table 4.2 Effect of treatments on growth performance of broiler chickens during grower-finisher period (days 22-42)

	Treatment					
	T1	T2	T3	T4	T5	P-value
Initial weight (g/b)	629.0 ± 7.07	636.0 ± 6.04	624.0 ± 4.47	632.0 ± 3.39	643.0 ± 8.28	0.27
ADG (g/b/d)	50.05 ± 0.75	51.10 ± 0.98	51.77 ± 0.57	50.28 ± 0.39	52.27 ± 0.86	0.26
ADFI (g/b/d)	102.75 ± 0.22	101.95 ± 0.47	102.98 ± 0.27	103.40 ± 0.32	102.65 ± 0.65	0.21
FCR	2.06 ± 0.03	2.00 ± 0.04	1.99 ± 0.02	2.06 ± 0.01	2.06 ± 0.05	0.42
Mortality (%)	0.00	0.00	0.00	0.00	0.00	-

Mean ± S.E., n = 5

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^8 cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide

[a, b] Means in the same row with unlike superscripts differed significantly (p<0.05)

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Table 4.3 Effect of treatments on overall growth performance of the broiler chickens during overall periods (days 0-42)

	Treatment					
	T1	T2	T3	T4	T5	P-value
Initial weight (g/b)	43.8 ± 0.32	43.4 ± 0.09	43.5 ± 0.00	43.6 ± 0.09	43.6 ± 0.09	0.41
Final weight (g/b)	1,755.3 ± 14.36	1,784.7 ± 25.07	1,789.4 ± 7.76	1,785.9 ± 20.51	1,798.6 ± 32.36	0.69
ADG (g/b/d)	34.19 ± 0.23	34.38 ± 0.43	34.44 ± 0.20	34.99 ± 0.34	35.35 ± 0.56	0.20
ADFI (g/b/d)	63.22 ± 0.13^{ab}	62.97 ± 0.20^{b}	63.55 ± 0.15^{a}	63.69 ± 0.18^{a}	63.77 ± 0.12^{a}	0.01
FCR	1.85 ± 0.01	1.83 ± 0.03	1.85 ± 0.01	1.82 ± 0.02	1.81 ± 0.03	0.55
Mortality (%)	0.00	0.00	0.00	0.87 ± 0.87	1.74 ± 1.07	0.21

Mean ± S.E., n = 5

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide

$^{a, b}$ Means in the same row with unlike superscripts differed significantly ($P < 0.05$)

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4.2 Qualitative and semi-quantitative examination of SE

4.2.1 Qualitative examination of SE

In Table 4.4, Qualitative examination of SE (number of positive plate/total plates) from IC (ileo-cecal content) samples did not significantly differ (P > 0.05) in days 15, 22 and 43 of age. However, IC samples from chicks in T4 and T5 groups at day 15 tended to have less positive plates than others and had similar positive plate as negative control (T1). Similarly, chicks in T5 group at day 22 (supplemented with YP+MOS) had lower positive plates (P > 0.05) than other groups. At day 43, there was no appearance examination of SE in all groups.

Qualitative examination of SE in LS (liver-spleen pools) in day 22 demonstrated that chicks in T3, T4 and T5 groups had slightly lower (P < 0.05) positive plates than positive control (T2) and were not significantly different from negative control (T1). It is apparent that although SE was not detected in the gut at day 43, some SE may be detected in LS pools as shown in T2 and T3 chicks at day 43. (Table 4.4)

4.2.2 Semi-quantitative examination of SE

In Table 4.5, the mean $\log_{10}$ number of SE per gram of IC samples from day 15 to day 43 was depicted. At day 15 of age, chicks in T4 had significantly lower SE count than positive control group and SE count was markedly reduced in T5 (P < 0.05) to be similar to negative control group. At day 22, SE count in all inoculated groups were decreased and it is shown that T4 and T5 chicks had lower SE count than T2 and T3 (P > 0.05) and were close to those count in T1. In comparison to SE count in LS samples, the SE count in LS samples demonstrated that T4 (SE+MOS) had the significantly highest SE count while T5 had the lowest SE count and the count was similar to negative control (T1). At day 22, SE count was only discovered in positive control chicks. The semi-quantitative examinations of IC and LS samples revealed that there was no SE detected at day 43 in all groups.

Table 4.4 Qualitative examination of SE (number of positive plates/total plates) in various treatment groups at different ages of chicks

Samples		Treatment					
		T1	T2	T3	T4	T5	P-value
IC	Day 15	1/10	3/10	3/10	2/10	1/10	0.78
	Day 22	2/10	7/10	6/10	4/10	2/10	0.22
	Day 43	0/10	0/10	0/10	0/10	0/10	-
LS	Day 15	1/15	3/15	1/15	3/15	2/15	0.62
	Day 22	$1/15^{b}$	$9/15^{a}$	$5/15^{ab}$	$6/15^{ab}$	$6/15^{ab}$	0.04
	Day 43	0/15	1/15	1/15	0/15	0/15	0.57

Treatments were CON: control; SE: *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide;

IC : Ileal-cecal content; LS : Liver-spleen

[a, b] Means in the same row with unlike superscripts differed significantly ($P < 0.05$)

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Table 4.5 Semi-quantitative examination of SE (log_{10} CFU/ml of SE) in various treatment groups at different ages of chicks

Samples		Treatment					
		T1	T2	T3	T4	T5	P-value
IC	Day 15	0.10 ± 0.10^{c}	5.10 ± 0.87^{a}	5.10 ± 0.40^{a}	2.50 ± 0.45^{b}	0.90 ± 0.19^{c}	< 0.0001
	Day 22	0.10 ± 0.10^{b}	1.50 ± 0.59^{a}	1.20 ± 0.30^{a}	0.50 ± 0.32^{ab}	0.70 ± 0.37^{ab}	0.09
	Day 43	0.00	0.00	0.00	0.00	0.00	-
LS	Day 15	0.13 ± 0.08^{b}	0.53 ± 0.23^{ab}	0.47 ± 0.31^{ab}	0.93 ± 0.19^{a}	0.13 ± 0.08^{b}	0.05
	Day 22	0.00	0.07 ± 0.07	0.00	0.00	0.00	0.43
	Day 43	0.00	0.00	0.00	0.00	0.00	-

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide;

IC : Ileal-cecal content; LS : Liver-spleen

$^{a, b}$ Means in the same row with unlike superscripts differed significantly (P < 0.05)

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4.3 Morphology and Immunohistochemistry study

4.3.1 Effect of treatments on Intestinal morphology

Table 4.6 demonstrated the intestinal morphology of duodenum in each period studied. At day 15, the negative control group had the highest villus height and was significantly greater ($P<0.05$) than all SE inoculated groups except T3. There were no significant changes in crypt depth and villus:crypt ratio (VC ratio). However, chicks in T2 tended to have the lowest VC ratio compared to other groups. The villus height in YP supplemented chicks (T3 and T5) were similar to negative control group at day 22 with T2 group having the shortest average villus height ($P<0.05$). The VC ratio was significantly lowest in positive control group (14 days after 2nd SE inoculation). All yeast supplemented groups had better VC ratio than T2. The best VC ratio was discovered in T1. There were no significantly differences in all morphology parameters among treatment groups at day 43.

The morphological parameters among groups during different days of age are demonstrated in Table 4.7. The villus height significantly increased when chicks grew except in T2 (SE group) that had slightly lower height than other groups at day 22. Additionally, the crypt depth were mostly increased at day 22 in all SE inoculated groups and slightly reduced at day 43. This resulted in the drop in VC ratio in T2 and T5 group at day 22 with the lowest VC ratio found in T2.

In Table 4.8, there were no significant differences in all parameter in jejunal segment of the intestine at day 15 of age. Likewise in duodenal part, the T2 chicks had significantly higher ($P < 0.05$) crypt depth at day 22 of age compared to other groups markedly lower depth ($P < 0.05$) in T3 and T5 that received YP. The VC ratio was also slightly lowest in T2 and slightly improved in inoculated groups. At day 43, T1 group had the best villus height and VC ratio and all inoculated groups (T2-T4) were not significantly different except the height in T3 group was slightly decreased. The crypt depth in T2 group was slightly higher than other groups.

Table 4.9, demonstrated that villus height in T4 ($P<0.05$) and T5 ($P>0.05$) had higher than other groups at day 22 and the chicks in T5 had slightly increase at day 43. The crypt depth in all groups except T1 and T3 were significantly increased at day 22 and 43 of the

study. The VC ratio also significantly dropped at day 22 in most groups in particular T2 and T5 groups when compared to day 15.

Villus height and crypt depth in ileal mucosa of chicks in T4 were significantly higher than the others especially T2 at day 15 with no difference in VC ratio (Table 4.10). At day 22, the highest crypt depth was found in T2 and T3 with the lowest VC ratio also discovered in T2 group. There was no significant change in all ileal mucosa parameter at day 43.

The parameter among groups of morphology of ileum in Table 4.11 demonstrated that villus height in T2, T3 and T5 significantly increased at day 22. The crypt depth was also higher ($P<0.05$) in T2 and T5 at day 22 compared to day 15. The VC ratio was significantly highest in T5 and slightly better in T4 at day 43 compared to day 15.

Table 4.6 Effects of treatment on intestinal morphology of Duodenum

		Intestinal measurement	Treatment					
			T1	T2	T3	T4	T5	P-value
Duodenum	Day 15	Villus height (µm)	1,154.00 ± 24.66^{a}	1,006.80 ± 51.54^{b}	1,068.80 ± 45.45^{ab}	975.20 ± 21.46^{b}	1,025.60 ± 15.10^{b}	0.02
		Crypt depth (µm)	72.80 ± 5.68	66.80 ± 5.46	67.60 ± 10.28	60.00 ± 0.89	62.40 ± 4.12	0.63
		Villus:crypt ratio	16.33 ± 1.55	15.73 ± 2.03	18.83 ± 5.05	16.27 ± 0.42	16.65 ± 0.83	0.92
	Day 22	Villus height (µm)	1,178.00 ± 47.20^{a}	987.20 ± 84.70^{b}	1,220.80 ± 54.07^{a}	1,124.80 ± 53.08^{ab}	1,184.00 ± 43.40^{a}	0.08
		Crypt depth (µm)	75.60 ± 5.53	107.60 ± 10.36	102.40 ± 6.88	95.60 ± 15.38	98.80 ± 6.09	0.20
		Villus:crypt ratio	16.09 ± 1.82^{a}	9.39 ± 0.86^{b}	12.07 ± 0.68^{ab}	13.51 ± 2.84^{ab}	12.07 ± 0.40^{ab}	0.09
	Day 43	Villus height (µm)	1,285.20 ± 74.17	1,268.80 ± 101.42	1,331.20 ± 87.12	1,160.00 ± 48.53	1,376.8 ± 66.94	0.39
		Crypt depth (µm)	79.60 ± 4.35	81.20 ± 10.86	78.00 ± 4.29	68.00 ± 8.07	80.00 ± 7.43	0.72
		Villus:crypt ratio	16.30 ± 1.17	16.32 ± 1.60	17.33 ± 1.61	17.85 ± 1.70	18.00 ± 2.20	0.92

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide;

$^{a, b}$ Means in the same row with unlike superscripts differed significantly (P<0.05)

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Table 4.7 Effects of treatment on intestinal morphology of duodenum (Comparison among different days of age)

		Treatment	Day 15		Day 22		Day 43		P-value
Duodenum	Villus height (µm)	T1 (CON)	1154.00	± 24.66	1178.00	± 47.20	1285.20	± 74.17	0.21
		T2 (SE)	1006.80	± 51.54^{b}	987.20	± 84.70^{b}	1268.80	± 101.42^{a}	0.06
		T3 (SE+YP)	1068.80	± 45.45^{b}	1220.80	± 54.07^{ab}	1331.20	± 87.12^{a}	0.04
		T4 (SE+MOS)	975.20	± 21.46^{b}	1124.80	± 53.08^{a}	1160.00	± 48.53^{a}	0.02
		T5 (SE+YP+MOS)	1025.60	± 15.10^{c}	1184.00	± 43.40^{b}	1376.80	± 66.94^{a}	0.0007
	Crypt depth (µm)	T1	72.80	± 5.68	75.60	± 5.53	79.60	± 4.35	0.66
		T2	66.80	± 5.46^{b}	107.60	± 10.36^{a}	81.20	± 10.86^{ab}	0.03
		T3	67.60	± 10.28^{b}	102.40	± 6.88^{a}	78.00	± 4.29^{b}	0.02
		T4	60.00	± 0.89^{b}	95.60	± 15.38^{a}	68.00	± 8.07^{ab}	0.07
		T5	62.40	± 4.12^{b}	98.80	± 6.09^{a}	80.00	± 7.43^{b}	0.004
	Villus:Crypt ratio	T1	16.33	± 1.55	16.09	± 1.82	16.30	± 1.17	0.99
		T2	15.73	± 2.03^{a}	9.39	± 0.86^{b}	16.32	± 1.60^{a}	0.02
		T3	18.83	± 5.05	12.07	± 0.68	17.33	± 1.61	0.30
		T4	16.27	± 0.42	13.51	± 2.84	17.85	± 1.70	0.31
		T5	16.65	± 0.83^{a}	12.07	± 0.40^{b}	18.00	± 2.20^{a}	0.03

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide

$^{a, b}$ Means in the same row with unlike superscripts differed significantly (P<0.05)

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Table 4.8 Effects of treatment on intestinal morphology of Jejunum

Intestinal measurement			Treatment					
			T1	T2	T3	T4	T5	P-value
Jejunum	Day 15	Villus height (µm)	546.72 ± 42.35	534.56 ± 30.75	631.52 ± 38.65	527.60 ± 73.26	535.36 ± 18.70	0.46
		Crypt depth (µm)	45.12 ± 2.99	46.56 ± 2.78	48.48 ± 9.81	39.20 ± 1.20	37.92 ± 3.02	0.50
		Villus:crypt ratio	12.34 ± 1.22	11.59 ± 0.78	15.95 ± 3.83	13.37±1.55	14.56 ± 1.49	0.60
	Day 22	Villus height (µm)	614.56 ± 40.36	602.72 ± 51.06	643.36 ± 46.46	690.24 ± 43.85	711.6 ± 55.18	0.44
		Crypt depth (µm)	55.04 ± 4.24^{c}	84.64 ± 3.56^{a}	60.8 ± 6.53^{bc}	71.36 ± 4.63^{ab}	68.00 ± 5.66^{bc}	0.006
		Villus:crypt ratio	11.30 ± 0.83	7.22 ± 0.80	11.37 ± 2.14	9.82 ± 0.79	10.72 ± 1.21	0.16
	Day 43	Villus height (µm)	910.40 ± 42.16^{a}	787.52 ± 24.54^{ab}	674.24 ± 58.54^{b}	764.00 ± 23.18^{ab}	787.60 ± 94.32^{ab}	0.09
		Crypt depth (µm)	57.20 ± 4.92	80.96 ± 3.40	61.60 ± 3.16	73.20 ± 9.83	68.80 ± 8.66	0.13
		Villus:crypt ratio	16.65 ± 2.15^{a}	9.80 ± 0.54^{b}	10.94 ± 0.76^{b}	11.01 ± 1.11^{b}	11.48 ± 0.40^{b}	0.005

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide;

$^{a, b}$ Means in the same row with unlike superscripts differed significantly ($P<0.05$)

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Table 4.9 Effects of treatment on intestinal morphology of Jejunum (Comparison with different days of age)

		Treatment	Day 15	Day 22	Day 43	P-value
Jejunum	Villus height (µm)	T1 (CON)	546.72 ± 42.35^{b}	614.56 ± 40.36^{b}	910.40 ± 42.16^{a}	0.0001
		T2 (SE)	534.56 ± 30.75^{b}	602.72 ± 51.06^{b}	787.52 ± 24.54^{a}	0.001
		T3 (SE+YP)	631.52 ± 38.65	643.36 ± 46.46	674.24 ± 58.54	0.82
		T4 (SE+MOS)	527.60 ± 73.26^{b}	690.24 ± 43.85^{a}	764.00 ± 23.18^{a}	0.02
		T5 (SE+YP+MOS)	535.36 ± 18.70^{b}	711.60 ± 55.18^{ab}	787.60 ± 94.32^{a}	0.04
	Crypt depth (µm)	T1	45.12 ± 2.99	55.04 ± 4.24	57.20 ± 4.92	0.13
		T2	46.56 ± 2.78^{b}	84.64 ± 3.56^{a}	80.96 ± 3.40^{a}	<0.0001
		T3	48.48 ± 9.81	60.80 ± 6.53	61.60 ± 3.16	0.37
		T4	39.20 ± 1.20^{b}	71.36 ± 4.63^{a}	73.20 ± 9.83^{a}	0.004
		T5	37.92 ± 3.02^{b}	68.00 ± 5.66^{a}	68.80 ± 8.66^{a}	0.006
	Villus:Crypt ratio	T1	12.34 ± 1.22^{ab}	11.31 ± 0.83^{b}	16.65 ± 2.15^{a}	0.06
		T2	11.59 ± 0.78^{a}	7.22 ± 0.80^{b}	9.80 ± 0.54^{a}	0.004
		T3	15.95 ± 3.83	11.37 ± 2.14	10.94 ± 0.76	0.34
		T4	13.37 ± 1.55	9.82 ± 0.79	11.01 ± 1.11	0.14
		T5	14.56 ± 1.49^{a}	10.72 ± 1.21^{b}	11.48 ± 0.40^{ab}	0.08

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide

Mean in the same row with different superscripts differed significantly ($P<0.05$)

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Table 4.10 Effects of treatment on intestinal morphology of Ileum

		Intestinal measurement	Treatment					
			T1	T2	T3	T4	T5	P-value
Ileum	Day 15	Villus height (µm)	326.56 ± 15.65^{bc}	293.12 ± 21.61^{b}	381.12 ± 27.16^{ab}	416.00 ± 34.55^{a}	325.12 ± 25.32^{bc}	0.02
		Crypt depth (µm)	35.52 ± 2.21^{b}	35.04 ± 3.05^{b}	42.40 ± 3.76^{ab}	47.68 ± 2.49^{a}	34.56 ± 2.25^{b}	0.01
		Villus:crypt ratio	9.30 ± 0.60	8.44 ± 0.35	9.35 ± 1.33	8.75 ± 0.67	9.50 ± 0.72	0.86
	Day 22	Villus height (µm)	439.84 ± 58.24	437.76 ± 28.07	585.12 ± 79.22	410.40 ± 39.18	491.04 ± 31.60	0.16
		Crypt depth (µm)	36.48 ± 4.36^{b}	61.76 ± 2.53^{a}	61.92 ± 5.52^{a}	42.72 ± 2.92^{b}	44.00 ± 2.44^{b}	0.0001
		Villus:crypt ratio	13.13 ± 2.72	7.20 ± 0.68	9.87 ± 1.84	10.03 ± 1.69	11.34 ± 1.07	0.22
	Day 43	Villus height (µm)	558.72 ± 25.35	607.20 ± 9.12	585.12 ± 21.15	571.04 ± 39.24	582.72 ± 19.08	0.72
		Crypt depth (µm)	44.96 ± 5.60	51.52 ± 1.76	48.24 ± 3.55	46.40 ± 3.84	46.72 ± 1.61	0.75
		Villus:crypt ratio	13.10 ± 1.61	11.86 ± 0.54	12.62 ± 1.68	12.45 ± 0.76	12.51 ± 0.47	0.96

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^8 cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide

Mean in the same row with different superscripts differed significantly ($P<0.05$)

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Table 4.11 Effects of treatment on intestinal morphology of Ileum (Comparison with different days of age)

		Treatment	Day 15	Day 22	Day 43	P-value
Ileum	Villus height (µm)	T1 (CON)	326.56 ± 15.65^{b}	439.84 ± 58.24^{b}	558.72 ± 25.35^{a}	0.003
		T2 (SE)	293.12 ± 21.61^{c}	437.76 ± 28.07^{b}	607.20 ± 9.12^{a}	<0.0001
		T3 (SE+YP)	381.12 ± 27.16^{b}	585.12 ± 79.22^{a}	585.12 ± 21.15^{a}	0.02
		T4 (SE+MOS)	416.00 ± 34.55^{b}	410.40 ± 39.18^{b}	571.04 ± 39.24^{a}	0.02
		T5 (SE+YP+MOS)	325.12 ± 25.32^{c}	491.04 ± 31.60^{b}	582.72 ± 19.08^{a}	<0.0001
	Crypt depth (µm)	T1	35.52 ± 2.21	36.48 ± 4.36	44.96 ± 5.60	0.27
		T2	35.04 ± 3.05^{c}	61.76 ± 2.53^{a}	51.52 ± 1.76^{b}	<0.0001
		T3	42.40 ± 3.76^{b}	61.92 ± 5.52^{b}	48.24 ± 3.55^{a}	0.02
		T4	47.68 ± 2.49	42.72 ± 2.92	46.40 ± 3.84	0.53
		T5	34.56 ± 2.25^{b}	44.00 ± 2.44^{a}	46.72 ± 1.61^{a}	0.004
	Villus:Crypt ratio	T1	9.30 ± 0.60	13.13 ± 2.72	13.10 ± 1.61	0.28
		T2	8.44 ± 0.35^{b}	7.20 ± 0.68^{b}	11.86 ± 0.54^{a}	0.0002
		T3	9.35 ± 1.33	9.87 ± 1.84	12.62 ± 1.68	0.35
		T4	8.75 ± 0.67	10.03 ± 1.69	12.45 ± 0.76	0.11
		T5	9.50 ± 0.72^{b}	11.34 ± 1.07^{ab}	12.51 ± 0.47^{a}	0.056

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^{8} cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide

Mean in the same row with different superscripts differed significantly (P<0.05)

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4.3.2 Effects of treatments on ileal IgA producing cells in lamina propria and mucosal line

Table 4.12 depicted the number of IgA positive cells in lamina propria and mucosal line in ileal mucosa. All SE inoculated groups had significantly higher positive cells than negative control group (T1) at day 15, 22 and 43. Chicks in T5 tended to have numerically higher IgA positive cells (P > 0.05) in all ages. The scale of IgA positive cells in mucoal line counted had highest in T5 group at day 15. At day 22 of age, chicks in MOS supplemented groups (T4 and T5) have higher level of IgA positive cells than others. There were no differences in scale of IgA positive cells in mucosal line than negative control at day 43.

Table 4.12 Ileal IgA producing cells in lamina propria and in mucosal line counts of broilers (cell/high power field)

IgA		Treatment					
		T1	T2	T3	T4	T5	P-value
Lamina propria	Day 15	2.23 ± 0.68[b]	13.47 ± 1.53[a]	13.57 ± 1.42[a]	12.17 ± 1.86[a]	15.93 ± 1.02[a]	<0.0001
	Day 22	5.60 ± 1.06[b]	18.50 ± 2.53[a]	18.20 ± 1.40[a]	19.30 ± 1.46[a]	19.90 ± 1.88[a]	<0.0001
	Day 43	7.80 ± 1.16[b]	23.23 ± 2.00[a]	24.57 ± 1.39[a]	24.70 ± 1.81[a]	25.40 ± 2.51[a]	<0.0001
Mucosal line*	Day 15	1	2	2	2	3	-
	Day 22	2	3	3	4	4	-
	Day 43	3	4	3	4	4	-

Treatments were CON : control; SE : *Salmonella* Enteritidis 10^8 cfu/ml; YP : Yeast protein extract; MOS : Mannan-oligosaccharide;

[a, b] Means in the same row with unlike superscripts differed significantly (P<0.05)

*IgA positive cells in mucosal line counted were: 1= 1-30, 2 = 31-60, 3 = 61-90, 4 = 91-120 and 5 = 121-150

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CHAPTER V

DISCUSSION

Starter period

In this study, *Salmonella* Enteritidis (S.E.) was administered by crop-intubation at days 1 and 7 of age. Two additives from yeast were examined singly (T3 and T4) and in combination (T5), the first one is yeast protein extract (YP) which was given only in the first 7 days of age as recommended without changing the crude protein level in the diet. The other additive is mannan-oligosaccharide (MOS) from yeast which basically helped to decrease attachment of Salmonella in the lumen of the gut and was included as the decline concentrations (2%, 1% and 0.5%) from day 8 to 42 of age. In comparison of all S.E. inoculated groups, the results clearly demonstrated in this period that the average daily gain (ADG) of the chick in T5 (YP+MOS) was the highest ($P<0.05$) compared to the positive control (T2). Moreover, chicks in T3 and T5 had better average daily feed intake (ADFI) than other groups. It is possible that this may be due to the glutamic acid and other ingredients in that the cell content of YP that can stimulate the palatability as it exerts savory flavors. Supplementation of MOS (T4 and T5) helped to reduce S.E. count as it can bind to type 1 fimbriae of the flagella of S.E. resulting in the excretion of MOS-S.E. complex to the excreta (Spring et al., 2000). Consequently this helped to slightly improve feed conversion ratio (FCR) in T5 group compared to others (Spring et al., 2000; Savage and Zakrzewska, 1997). MOS was indigestible in small intestine; therefore, can pass through to the large intestine and provides competitive binding sites for these intestinal pathogens (Ofek et al., 1977). The S.E. that was binding with MOS cannot colonize and was excreted out of the host and this decreased quantity of S.E. in the lumen (Spring et al., 2000). It is demonstrated that the qualitative examination of S.E. (number of positive plate per total plate) from IC (Ileo–cecal content) samples from chicks in T4 (MOS) and T5 (YP+MOS) groups at day 15 tended to have less positive plates than others and similar positive plate as negative control (Table 4.4). Similarly, the quantitative examinations (Table 4.5) of IC from the chicks in T4 and T5 groups at day 15 have significantly less than positive control. In addition, T5 groups had less positive

plates than T4 group (MOS alone). The YP consisted of more beneficial nutrients. Rutz et al. (2004) reported that YP supplementation in the starter period resulted in the higher feed intake and may benefit early enteric development of young poultry, helping to resist enteric disease challenged and maintained epithelium integrity. This created the effective barrier to prevent the spread to target organs e.g. liver and spleen (Andreas et al., 2000). It is demonstrated that qualitative examination of S.E. from LS (liver – spleen pools) samples in T3 (YP) and T5 (YP+MOS) groups in day 15 were slightly less than the positive control group. Similarly, the quantitative examination of LS samples in day 15 had lower positive plates ($P>0.05$) than other groups (Table 4.5). The infection of S.E. leads to changes in intestinal morphology such as shorter villi and deeper crypts causing by lipopolysaccharide (LPS) toxin (Xu et al., 2003). These resulted in changed morphology of intestine and had influence on the digestibility and efficiency to protect from pathogen. The morphology of duodenum at day 15 showed the highest villus height and was significantly greater ($P<0.05$) than all S.E. inoculated groups except T3. There were no significant changes in crypt depth and Villus:Crypt ratio (VC ratio). Similarly, in Tables 4.8 and 4.10, villus height in jejunum and ileum from T3 was slightly higher than positive control ($P>0.05$.) Iji et al. (2001) reported that MOS affected intestinal morphology and function. The synergistic effect of both YP and MOS was demonstrated in the chicks from T5 group which had the highest VC ratio of ileal mucosa compared to the positive control.

Grower and finisher period (22-42 days)

There was no significant difference in initial weight, ADG, ADFI, FCR and percentage of mortality. However the FCR of chicks in T4 was inferior to T2 although it was not significant different. This finally was consistent to Eren et al. (1999) who reported no significant differences in FCR. The effected of MOS was demonstrated on S.E. contamination. Chicks in T4 and T5 group at day 22 had lower positive plates than positive control (qualitative examination of S.E.).

The semi-quantitative examinations of IC and LS sample revealed that there was no SE. detected in T1, T4 and T5. The nil determination of SE. at the slaughter age is the

utmost important benefit of additive used in broiler. Supplementation of MOS alone or MOS+ YP helped to prevent organ colonization of SE. The villus height of duodenum groups T3 and T5 increased when compared to positive control. Moreover VC ratios from the supplemented groups were slightly better than positive control. The intestinal villus and crypt morphology in chickens were directly relevant to the functional intestine and the growth of chicken (van Dongen et al., 1976). The villus height of duodenum in YP supplemented chicks (T3 and T5) were as similar to negative control groups at day 22 with T2 group have the shortest average villus height. All yeast supplemented groups had lower crypt depth and better VC ratio than T2. It is possible the SE. directly caused damage to the villi and decrease the villus height; resulted in the shorter villi when compared to non-inoculated control chicks (T1). The crypts contain stem cells that can proliferate and differentiate to the new mature cells to the top of villus, mainly absorptive, goblet and endocrine cells (Van Dongen et al., 1976). It is demonstrated that the chicks from SE. group had shorter villus height and deeper crypt depth than the naive chicks at day 22. This supports the previous finding on the detrimental effect of SE. on intestinal integrity. YP supplement helped to restore the villus height as well as its effect in reducing crypt depth, thus, improving cell turnover.

In the overall period, chicks in T5 (YP+ MOS) had better growth performance than other groups. In qualitative and semi-qualitative examination of SE. in IC content, the chicks from T4 and T5 (both groups were MOS supplemented) groups showed less positive appearance of S.E. than positive control group.

Mucosal immunity is an important part of humoral immunity and secretory IgA is the effective component of mucosal immunity. It is the most prominent antibody present at mucosal surfaces, and provides passive immune against invading pathogens in the gastrointestinal tract. In this study, chicks in SE. inoculated groups had significantly higher IgA positive cells than negative control group (T1) at days 15, 22 and 43. Chicks in T5 tended to have slightly higher IgA positive cells ($P > 0.05$) in all ages. Similar to the previous study, birds fed mannan oligosaccharide supplemented diets had greater IgA content in the duodenum (Gao et al., 2008). This implies that it may stimulate the humoral immune system to produce more antibodies. Increased antibodies cover the

surface of intestinal mucosa and can protect villus from damage. This could be partly responsible for the changes in intestinal morphology in this study. The intestine is one of the organs subject to contact with exotic pathogens and toxins. Secretory IgA can function in eliminating antigens from tissues via immune complex formation (Robinson et al., 2001).

Oligosaccharides containing mannose have been shown to affect the immune system by stimulating liver secretion of mannose-binding protein. This protein, in turn, can bind to bacteria and stimulate the complement cascade of the host immune system (Janeway, 1993; Newman, 1994). The mucosal line on the surface of the intestinal epithelium is the first barrier to enteric infection. Thus, the number of IgA positive cell on mucosal line, is an important feature to protect against pathogens. The IgA positive cells on mucosal line counts were highest in T5 group at day 15. At day 22 of age, chicks in MOS supplemented groups (T4 and T5) also elicited higher scale than others. Invading bacteria stimulated innate immune system to develop and recognize key molecular structures of pathogen, including lipopolysacchharides, peptidoglycans, and possibly the mannose structures in the cell walls of yeasts (Ferket et al., 2002). In previous studies, MOS has been shown to have a positive influence on immunoglobulin. Savage et al. (1996) reported an increase in plasma IgG and bile IgA in chicks fed diets supplemented with 0.11% MOS although the current study had no significant differences. Verduzco et al. (2009) showed that MOS increased mucosal concentrations of intestinal IgA, Swanson et al., 2002 reported an increased IgA levels in the ileum in dogs fed diets supplement with MOS. An increase in antibody response to MOS would be expected because of the ability of the innate immune system to react to foreign antigenic material of microbial origin. The compounds of MOS and pathogen would bind to macrophage reception sites and recognizing specific sugars found in glycoprotein of the epithelial surface, the activating cascade reaction through to the macrophages and release cytokines, therefore triggering the immune response (Collet, 2000). Therefore this result support the finding that MOS increased protective IgA response and improve resistance to pathogen (Silva, 2000, Rutz et al., 2004).

In conclusion, the results of this study demonstrated that YP and MOS supplementation helped to decrease and prevent *Salmonella* Enteritidis colonization in LS pool and IC content of SE. inoculated chicks. YP supplementation improved the intestinal morphology by increase villus height, decrease crypt depth and improve VC ratio compare to other groups. MOS supplementation reduced amount of SE. and helped to improve intestinal integrity. Salmonella inoculation increased IgA producing cells in ileal mucosa. Supplementation of both additives had no additional positive effect compare with positive control. There was an increase in scale of IgA positive cells on mucosal lining in MOS supplemented groups.

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Swanya Siribopitr, Indhira Kramomthong, Noppadon Pirarat and Kris Angkanaporn. 2010. Effects of protein extract and mannan-oligosaccharide from yeast on growth performance, Salmonella contamination and intestinal morphology of broilers challenged with *Salmonella enterica* serovar Enteritidis. In proceeding of The 6th Journal of Science and Technology Mahasarakham University : Roles on September 9-10, 2010 at Taksila Hotel, Mahasarakham, Thailand.

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