EVALUATION OF DIETARY NUCLEOTIDES FOR BROILERS

by

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ABSTRACT

Nucleotides are the basic units that make up nucleic acids, such as deoxyribonucleic acd (DNA) and ribonucleic acid (RNA). Nucleotides are involved in many biological processes. They are important in nucleic acids (e.g. DNAs and RNAs) synthesis and as a source of cellular energy. They affect the immune system and are involved in physiological and morphological changes in small intestinal development. Nucleotides are not considered an essential nutrient because they can be synthesized by a *de novo* pathway from amino acid precursors and recycled through a salvage pathway. However, these mechanisms may not always be adequate to supply nucleotides for an organism during times of stress. Stressors which may impact nucleotide requirements include high stocking density, unsanitary conditions, disease challenges, and recovery from injuries. A series of experiments were conducted to determine the effect of the dietary nucleotides on the performance and development of the gastrointestinal tract (GIT) of broilers under certain stress conditions. In the first experiment, *Torula yeast* RNA (as a source of dietary nucleotide supplementation) and a commercial nucleotide product (Nupro®) were fed to broilers raised under normal conditions and environmental stressed conditions; previously used litter. Body weight gain, feed intake, feed conversion ratios, mortality, and physical and morphological development of the gastrointestinal tract were measured. Supplementing the diet

with dietary nucleotides had no effect on performance of broilers under normal conditions; however, dietary nucleotide supplementation improved performance of birds reared in previously used litter. In the second experiment, the effect of *Torula yeast* RNA supplementation on performance, intestinal tract development and morphology of broilers challenged with a high-dose Coccivac-B® vaccine and reared in an increased stocking density were determined. Supplementing the diets with *Torula yeast* RNA had improved performance of birds challenged with a high-dose Coccivac-B® vaccine and reared in the increased stocking density conditions. In the third experiment, two dietary nucleotides (*Torula yeast* RNA and Nupro®) and a nucleotide precursor (glutamine) were fed to broilers challenged with Coccivac-B® vaccine. Broiler performance and nutrient utilization (nitrogen corrected apparent metabolizable energy; AME_N) were measured. Dietary nucleotide (0.25% *Torula yeast* RNA and 2% Nupro®) supplementation was effective at alleviating the negative effects of *Coccidiosis* vaccine challenge. The increased AME_N value of diets with supplemental levels of *Torula yeast* RNA and Nupro® resulted in increased growth performance. However, the supplementation of a nucleotide precursor, glutamine, did not replace nucleotides in broilers. In the fourth experiment, *Torula yeast* RNA was added to semi-purified diets containg high levels of casein or crystalline amino acids mixture of broilers challenged with Coccivac-B® vaccine. Performance and relative weight, morphology, and gross lesion score in small intestine of birds were measured. Birds fed semi-purified diets containing high levels of casein or crystalline amino acids mixture had very low feed intake and slow growth rate. The 25 × dose Coccivac-B® challenge did not affect performance and the supplementation of 0.25% *Torula yeast* RNA to the semi-purified diets had no an effect on the performance of birds. Thus, nucleotides do not appear to be an essential nutrient without sufficient stress conditions. Overall, nucleotides are not an essential nutrient

under normal conditions. However, the dietary nucleotide supplementation may be important to maintain maximum growth and performance when birds are exposed to stress conditions.

INDEX WORDS: Nucleotides, Performance, Stress conditions

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DEDICATION

This thesis is dedicated to my parents, and grandmother for their endless encouragement, and especially to my wife Soyoung for sincerity, patience, strength, and trust to do my best.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Nucleotides are the basic units of nucleic acids, such as deoxyribonucleic acd (DNA) and ribonucleic acid (RNA). Nucleotides are involved in many biological processes including nucleic acid synthesis, cellular energy metabolism (e.g. ATP and GMP), and mediation of hormonal action (cAMP) (Cosgrove M, 1998). In addition, nucleotides have important effects on the immune system and small intestinal growth and development (Yu, 1998). Nucleotides can be synthesized by a *de novo* pathway as well as recycled through a salvage pathway. However, some studies have indicated that both mechanisms may not always supply enough nucleotides for animals under certain conditions such as reproduction, environmental stress, disease challenge, or recovery from injuries (Carver and Walker, 1995). Thus, Yu (1998) suggested that nucleotides are "semi" or "conditionally" essential nutrients in animals.

An external stress on birds can be categorized as a biological or environmental challenge depending on its source of insult. For example, high stocking density and a *Coccidiosis* challenge may lead to biological changes in bird. High stocking density can decrease broiler performance and change small intestinal morphology, such as crypt depth and villous height. These changes may be due to reduced airflow between birds and reduced access to feed and water and increased number of infectious coccidia oocysts in the litter (Burkholder *et al.*, 2008; Puron *et al*., 1995; Lillehoj and Lillehoj, 2000). *Coccidiosis* is a parasitic disease of the intestinal tract of animals, caused by several coccidian protozoa. Coccidian parasites infect the intestinal tract of animals. Multiple species of *Eimeia* are involved in the *Coccidiosis* infections. Three species of *Eimeria*; *E. acervulina*, *E. maxima*, and *E. tenella*, have been diagnosed in poultry (McDougald *et al*., 1997). *E. acervulina* infection causes damage in the upper parts of the small intestine such as

duodenum, whereas *E. maxima* and *E. tenella* infect the jejunum and ceca, respectively. *Coccidial* infections lead to an increase in mortality, diarrhea, bloody feces, reductions in weight gain, poor feed efficiency, reduced nitrogen corrected metabolizable energy (ME_N) and amino acid (AA) digestibility in chicks (Williams, 2002; Persia *et al*., 2006). *E. acervulina* infections lead to several gastrointestinal morphological changes; mucosal soughing, truncated and thickened villous, and increased crypt depth and epithelial cell turnover, inflammation, increased relative length and weight of intestine, reduced performance and nitrogen corrected metabolizable energy (AME_N) in chicks (Allen, 1984; Allen, 1987; and Persia *et al*., 2006). Therefore, we hypothesized that a *Coccidiosis* challenge and high stocking density would reduce performance and alter intestinal morphology of birds during the starter period when the digestive tract undergoes rapid development.

Little is known about dietary nucleotides and their role in performance, intestinal development, inflammation, and nitrogen nutrient utilization in birds that are stressed. Thus, the conducted studies included a series of four experiments with the following objectives;

1) To determine the effect of a dietary nucleotide source, *Torula yeast* RNA, and a commercial nucleotide product (Nupro®) on performance and physical and morphological development of the gastrointestinal tract in broilers rasied under normal condition or environmental stress conditions (previously used litter).
2) To determine the effects of a dietary nucleotide supplementation on performance, intestinal tract development and morphology of broilers challenged with Coccivac-B® vaccine combined with an increased stocking density.
3) To determine the effects of two dietary nucleotide products, *Torula yeast* RNA and Nupro®, and a nucleotide precursor (glutamine) on performance and nutrient utilization

(nitrogen corrected apparent metabolizable energy; AME_N) in broilers challenged with Coccivac-B® vaccine.

4) To determine the effects of *Torula yeast* RNA supplementation on performance and on the relative weight, morphology, and gross lesion scores in the small intestine of Coccivac-B® vaccine challenged birds fed semi-purified diets containing high levels of a casein or high levels of a crystalline amino acids mixture.

LITERATURE REVIEW

NUCLEOTIDES

Nucleotides are the basic units of nucleic acids (e.g. DNAs and RNAs). They are needed for reproduction and multiplication of living cells (Cosgrove, 1998). For years, nucleotides were not considered an essential nutrient for use in any dietary programs in animals and humans. It was thought that animals were able to produce sufficient nucleotides to meet their physiological demands via a *de novo* synthesis or a salvage pathway (Hoffmann, 2007). The salvage pathway is the re-synthesis of nucleotides from nucleosides that originate from nucleotide catabolism or dietary sources. In other words, the salvage pathway is recycling nucleotides from dead cells. However, over the past several years, studies have indicated that both mechanisms may not always provide enough nucleotides under certain conditions. In addition, dietary nucleotides may become essential when the endogenous supply is insufficient during biologically active situations: rapid growth, reproduction, environmental change or challenge (high stocking density and used litter conditions), protein-energy malnutrition and nutritional deficiency, combating disease, and recovery from injury. Thus, nucleotides are considered "semi" or "conditionally" essential nutrients in animals (Yu. 1998).

1. Structure

Nucleotides contain three major components: a sugar (either ribose or deoxyribose), a nitrogen base (either a purine or a pyrimidine) and one or more phosphate groups (Figure 1.1). When the phosphate group is absent, the compound is called a nucleoside. Thus, a nucleoside is composed of the base linked to a sugar in N-glycoside linkage (Figure 1.2). Nucleic acids are

polymers made up of nucleotides. The base of a nucleotide is joined covalently (at N-1 of pyrimidines and N-9 of purines) in an N-glycosyl bond to the 1' carbon of the pentose. The nucleic acids are covalently linked through the phosphate group, in which the 5'phosphate group of one nucleotide unit is joined to the 3'-hydroxyl group of the next nucleotide, creating a phosphodiester linkage. Nucleic acids have two kinds of pentose. If the sugar is ribose, they are components of ribonucleic acid (RNA), and if the sugar is deoxyribose, they are components of deoxyribonucleic acid (DNA). DNA contains two major purine bases, adenine (A) and guanine (G) and two major pyrimidines, cytosine (C) and thymidine (T). RNA contains adenine (A) and guanine (G), cytosine (C), and uradine (U). A nucleoprotein is any protein that is linked to a nucleic acid and found in the nuclei and cytoplasm of all living cells, as in chromatin anc ribosomes (Voet and Voet, 2004).

2. Biosynthesis

Nucleotides and nucleic acids are continuously being formed and degraded. There are two major pathways for a formation of nucleotide or nucleic acids: *de novo* synthesis and a salvage pathway. Nucleotides can be synthesized within cells by a *de novo* pathway from amino acid precursors, such as glutamine, formate, glycine, and aspartic acid. Also, they can be formed through a salvage pathway. In the salvage pathway, purine and pyrimidine are formed from intermediates from the degradative pathway of nucleotides. Salvage pathways are also used to recover bases and nucleosides are synthesized from degradation of RNA and DNA. This salvage pathway is simpler and more energy efficient than the *de novo* synthesis of nucleotides and is modulated by the availability of free bases. The salvage pathway is especially important in some organs such as the intestinal tract and bone marrow, where nucleotides synthesis capacity is

limited. However, nucleotides supplied via the aforementioned mechanisms may not be sufficient to maintain needs during rapid growth, nutritional deficiency, combating diseases and recovery from injuries status (Cosgrove, 1998). Thus, dietary nucleotides may become an essential nutrient for optimal function in the body when the endogenous supply is insufficient (Yu, 1998; Carver, 1999).

3. Sources of nucleotides

Most feed ingredients contain nucleoproteins (proteins conjugated to nucleic acids), which serve as dietary sources of nucleotides (Mateo, 2005). Thus, it is a challenge to formulate animal diets that are nucleotide free. Mateo (2005) measured the nucleotide concentrations in some commonly used feed ingredients (Table 1.1). The total nucleotide content of non-fat dried milk, dried whey, and whey protein were 366, 294, and 282 ppm, respectively, and those ingredients contained a high level of total nucleotides compared to other feed ingredients the author measured. The major ingredients for poultry diets, which are corn and soybean meal, contained 9 and 38 ppm total nucleotides, respectively (Table 1.1). Organ meats, fish, and poultry are rich in nucleotides (Clifford and Story, 1976; Barness, 1994). Among organ meats, animal liver had higher total purine and RNA content than heart and brain, and particularly, adenine level in liver was much higher than the levels in other organs (Table 1.2). Among fresh seafood sources, guanine level in anchovies and sardines was much higher than its level in squid (Table 1.3). The nucleotide content was also high in ingredients such as animal protein soluble, fish meal and fish by-product, legumes, yeast extracts, and single cell proteins (SCP) ; Table 1.2 and 1.3 (Deveresse, 2000; Ingledew, 1999). In addition, dry whole yeast used in bakers and brewers industry had a high nucleic acid content ranging from 3.9 to 9.5% (Maloney, 1998).

Carver and Walker (1995) mentioned that the level of nucleotides, especially inosine monophosphate (IMP), is related to protein level on the foods. That is, foods containing high levels of protein have high nucleotide content with the exception of soybean meal. Soybean meal and corn, oil, oilseeds, and muscle protein, which is mainly composed of actin-myosin protein molecules, contain low levels of nucleotides (Deveresse, 2000). Fruits, vegetables, and dairy products also are poor sources of dietary nucleotides (Barness, 1994). Human milk especially during early lactation contains relatively high nucleotide levels. The concentrations of nucleic acid, nucleotides, and nucleosides was 68 ± 55μmol/L, 84 ± 25μmol/L, and 10 ± 2μmol/L, respectively in human milk (Johke, 1963). Human milk contains approximately 25% of its nitrogen as non-protein nitrogen including substances such as urea, free amino acids, nucleic acids, creatine, nucleotides and carnitine. Free and cellular nucleotides, mainly cytidine 5'monophosphate (CMP) and adenosine 5'monophosphate (AMP), account for 2 - 5% of the non-protein nitrogen in human milk (Cosgrove, 1998).

4. Nucleotide absorption and metabolism

Dietary nucleoproteins and nucleic acids are broken down by proteases and nucleases to nucleotides. The phosphate in nucleotides is cleaved by alkaline phosphatases and nucleotidases in the small intestine to form nucleosides (Carver and Walker, 1995) (Figure 1.3). Nucleosides are absorbed better in the intestinal lumen than nucleotides because nucleotides have negatively charged phosphate groups that blocked absorption (Yu, 1998). Nucleotides also have a limited capacity to pass through cell membranes under physiological conditions because of the lack of a transporter system (Sanderson and He, 1994). Nucleosides uptake is the major pathway for the entering of bases (purines and pyrimidines) into the epithelial cells in the intestine and then

partially metabolized. More than 90% of dietary and endogenous nucleosides and purine and pyrimidine bases are absorbed into the enterocyte (Uauy *et al*., 1990). Most of these absorbed nucleosides and purine and pyrimidine bases are rapidly degraded into uric acid and allantoin within the enterocytes (Sonoda and Tatibana 1978). In particular, purine nucleotides are degraded into uric acid, while pyrimidine nucleotides are degraded to *β*-alanine and *β*-amino iso-butyric acid (Carver and Walker, 1995).

5. Functions of nucleotides

Energy source

Adenosine 5'triphosphate (ATP) is the most abundant nucleotide in the cell (Carver and Walker, 1995). ATP is an energy-rich precursor that transports chemical energy within cells for metabolism (Cosgrove, 1998). Three high-energy phosphate bonds in an ATP molecule are the key that allows ATP to serve as a general currency of energy in biological systems (Voet and Voet, 1995). Consequently, ATP plays important roles in the active transportation of molecules and ions, macromolecules synthesis and mechanical work at the cellular level. Extracellular adenosine stimulates glucose production in the perfused rat liver (Buxton *et al.* 1986) and induces System A amino acid transport in cultured rat hepatocytes (Kiyokawa *et al.* 1991). In addition, it works as a phosphate donor for nucleotide synthesis and allosteric effectors in a variety of metabolic pathways (Carver and Walker, 1995). Therefore, nucleotides especially, ATP are an important energy source in the body.

Constituents of coenzyme and biological regulators

Nucleotides and their derivatives such as, flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$), and coenzyme A (CoA) play an important role in many biosynthetic pathways as intermediates and are involved in the synthesis and oxidation of fatty acid and oxidation of pyruvate in the citric acid cycle. All of these nucleotide derivatives are also important components in carbohydrate, protein, fat and nucleic acid metabolism (Cosgrove, 1998; Carver and Walker, 1995). For instance, uridine diphosphate (UDP) is a major carrier of sugar residues in the biosynthesis of polysaccharides and uridine diphosphate glucose (UDP-glucose) is used as a substrate for a donor of glucose. Uridine diphosphate is also an intermediate in the enzymatic biosynthesis of glycogen. Furthermore, adenosine 3', 5'-cyclic phosphate (cyclic AMP) plays a key role in regulating biological processes in all forms of life. The cAMP is synthesized from ATP by the adenylate cyclase action, and increased by adrenaline and glucagon (Cosgrove, 1998). Thus, nucleotides and the derivatives of nucleotides are of importance in metabolisms of fatty acid, carbohydrate, and protein.

Protein synthesis

Several studies have been conducted on the effects of dietary nucleotides on protein synthesis. Whole body protein turnover in rats receiving total parenteral nutrition supplemented with nucleotides increased as compared to rats receiving nutrition without of nucleotides supplementation, when the rats had been 70% hepatomized (Iwasa *et al*., 1997). Walsh *et al*. (1990) suggested that dietary nucleotides might regulate the synthesis of specific proteins that promote the transcription of hypoxanthine guanine phosphoribose transferase, which is a key

enzyme for purine salvage. The authors also found that nucleotides are important for stimulating ornithine decarboxylase, adenosine deaminase, and intestinal RNA content, effecting cell growth and proliferation. Yamauchi *et al*. (1998) also reported that mice that received intraperitoneal administration of a well-balanced nucleoside-nucleotide mixture had increased small intestinal RNA levels, which indicated an increased proliferation of enterocytes as compare to the mice fed the free-nucleotide diet. Thus, nucleotides are important to increase body protein turnover rate, specific proteins synthesis, and enterocyte proliferation.

Lipid metabolism

Several studies have been conducted on the effects of dietary nucleotides on lipid metabolism. Gil *et al*. (1986) measured plasma fatty acid levels and erythrocyte membrane phospholipids composition in infants receiving either nucleotide-supplemented formula with breast-feeding or a non-supplemented nucleotide formula. The authors found that plasma long-chain polyunsaturated fatty acids (PUFA) of the n-6 series greater than 18 carbon atoms increased in infants that received nucleotide-supplemented formula compared to infants fed non-supplemented nucleotide formula. Cosgrove (1998) suggested the mechanism at work here may be that nucleotides cause changes in the intestinal flora that may affect long-change PUFA levels. Carver and Walker (1995) suggested that dietary nucleotides have a role in the conversion of 18 carbon essential fatty acids to 20 and 22 carbon long chain polyunsaturated fatty acids by facilitating the increase in length of the carbon chain. Therefore, dietary nucleotides may increase omega-3 and omega-6 polyunsaturated fatty acid in animal tissues.

Amino acid precursor effects

Nucleotides are synthesized from amino acids such as glutamine, glycine, formate and aspartic acid within cells. The precursor of nucleotides, glutamine, is the principal metabolic fuel in small intestinal enterocytes, lymphocytes, macrophages, and fibroblasts (Cynober, 1999; Andrews and Griffiths, 2002). Glutamine is also considered an essential amino acid in some species under inflammatory conditions including infection and injury (Newsholme, 2001). A number of studies indicated the advantage of glutamine supplementation on GI tract development, performance, and immunity in animals. For example, supplementation of glutamine in the diet resulted in increased cytokine production as well as lymphocyte proliferation in rats (Reynolds *et al*., 1988). The addition of 1% glutamine increased body weight (BW) gain, feed conversion ratios, and intestinal villous height of turkey poults during the first week of age (Yi *et al*., 2001). Also, chicks fed diets with 1% glutamine supplementation had heavier intestinal relative weights and longer intestinal villi. In the same study, chicks fed diets supplemented with 1% glutamine for 21 d had higher bile, intestinal, and serum IgA and serum IgG concentration (Bartell and Batal, 2007). Therefore, a dietary nucleotide precursor, such as glutamine, supplementation to the poultry diets appears to improve GI tract development, growth performance, and immune response.

6. Effects of nucleotides

Effects on the gastrointestinal tract

Dietary nucleotides affect growth and development of intestinal epithelium, which is a rapidly proliferating tissue with a high cell turnover rate. Nucleotides appear to stimulate the development of the intestinal lining and intestinal enzyme concentrations in animals. They also

have beneficial effects on recovery from intestinal injuries caused by malnutrition or chronic diarrhea (Uauy *et al*., 1990; Ortega *et al*., 1994; Nunez *et al*., 1990; Bueno *et al*., 1994). For example, rats fed a diet supplemented with 0.8% nucleoside mixture (as a stable precursor of nucleotides) had increased disaccharide activities and villous height in the gut as compared to the rats fed a control diet without nucleoside supplementation (Uauy *et al*., 1990). Ortega *et al*., (1994) reported that the intestinal mucosa of birds fed a diet supplemented with dietary nucleotides after feed deprivation recovered faster indicated by increased mucosal weight and protein content. A diet supplemented with nucleotides also increased DNA and protein content, and increased disaccharidase activity in intestine, as well as, increased villous height in rats with lactose-induced chronic diarrhea or malnutrition (Nunez *et al*., 1990; Bueno *et al.*, 1994). The positive effect on the gastrointestinal tract in animals fed the diet supplemented with dietary nucleotides may be due to enhanced DNA and RNA synthesis by increasing the nucleotide pools. This increased DNA and RNA synthesis enhances growth and differentiation of the enterocytes after injuries or malnutrition. Therefore, an exogenous supplementation of nucleotides may help optimize tissue function in the GI tract and stimulate activity of brush border enzymes when the endogenous supply of nucleotides might be limited by mucosal injury.

Effects on the immune system

Dietary nucleotides have been reported to play a crucial role in the immune system, such as lymphocyte activation (Carver and Walker, 1995; Cosgrove 1998). Lymphoid tissues defend the body against infections by the action of white blood cells, the most numerous being the lymphocytes. Because immune cells like lymphocytes are continuously produced and released into circulation to detect infection, adequate nucleotide supplementation is required in lymphoid

tissues (Uauy *et al*., 1990). Also, dietary nucleotides play a beneficial role to defend against bacterial or fungal infections by increasing host resistance to such infections (Kulkarni *et al*., 1986). A number of studies indicated the effects of the nucleotide supplementation in diets on regulating immune response in animals and humans. Nagafuchi *et al*. (1997) suggested that dietary nucleotides may influence the level of serum antibody isotypes, down-regulate the Th2 immune response and up-regulate the Th1 immune response. The authors found that BALB/c weanling mice fed diets supplemented with nucleotides had decreased serum IgE level and splenic inteleukin-4 (IL-4; a cytokine produced by Th2) and increased interferon-γ (INF- γ; a cytokine produced by Th1) levels. Carver *et al*. (1991) found that infants fed nucleotide-supplemented milk had an increase in natural killer cell activity and IL-2 production, which are the body's natural response to microbial and viral infections or tumor control. Supplementing diets with nucleotides enhanced the survival of animals from an infectious diseases challenge. For example, Kulkarni *et al*. (1986) reported that 0.25% purified RNA supplementation decreased mortality rate from *Staphylococcus* or *Candida albicans* challenge in induced septic mice. The mechanism of immune stimulation by dietary nucleotides supplementation is unclear. However, Van Buren *et al*., (1985) suggested that the effects of dietary nucleotides on cellular immune responses may be due to acting on the T-helper cell mediated effects by increasing antigen processing or lymphocyte proliferation. Even though the mechanism of immune stimulation by the dietary nucleotides are not completely known, dietary nucleotides may be of importance for optimal function of the cellular immune response such as, increased INF-γ, decreased IL-2 production and natural killer cell, and increased resistance to bacterial or fungal infection.

7. Previous studies

Human studies

Supplementation of dietary nucleotides to infant formula is beneficial to intestinal microflora and reduces diarrhea (Yu, 1998). For example, when infants were fed formula supplemented with nucleotides, infants had significantly lower incidence of diarrhea as compared to the infants who did not receive the nucleotide supplementation (Cosgrove, 1998). This may be explained partially by role of dietary nucleotides in controlling intestinal microflora in infants by increasing bifidobacteria. Gil *et al*. (1986) found that the infants fed nucleotide supplemented commercial formula had higher concentrations of bifidobacteria in their feces as compared to the infants receiving a commercial formula.

Swine studies

Dietary nucleotide supplementation to swine diets has positive effects on performance, physiological and morphological development of intestine, and also reduces the incidence of diarrhea. For example, Andres-Elias *et al*. (2007) studied a nucleotide rich yeast extract (containing 25% free nucleotides) and carob pulp as possible alternatives to antimicrobial growth promoters for newly weaned pigs. There were no significant effects of dietary treatment on the performance and the histological parameters (villous height and width, crypt depth, and small intestine relative weight) in weaned pigs due to the addition of 0.15% nucleotide-rich yeast extract to the diets. However, in another study, Maribo (2003) found improved performance due to the dietary supplementation of a yeast extract (Nupro®; containing 7% total nucleotide content) as a source of nucleotides in swine infected with Escherichia (*E.) coli*. The addition of the yeast extract to the swine diet at 4.0% resulted in improved BW gain, reduced incidence of

diarrhea, and improved feed conversion ratios. In addition, dietary nucleotide supplementation may influence the development of intestinal microflora. Mateo (2005) reported that nucleotide supplementation in pig diets decreased concentration of potentially pathogenic bacteria (i.e., *Cl. Perfringens*) and increased beneficial bacteria (i. e., *L. acidophilus* and *Bifidobacterium spp*.) in feces. The author suggested that the mechanism by which nucleotides affect the intestinal microflora may be due to a decrease in the luminal pH in the intestine. Metabolites such as, acid and bacteriocins produced by *Bifidobacterium spp*. may cause a decrease in the pH in the gastrointestinal tract that could inhibit the growth of *Cl. Perfringens* over a period of time. Thus, dietary nucleotide supplementation in swine diets increases performance, changes physiological and morphological development of the intestine and reduces incidence of diarrhea.

Poultry studies

Supplementation of dietary nucleotides in poultry diets affects performance and lymphoid organs. Studies have been conduced to test the effects of dietary nucleotides, commercial nucleotide products and/or yeast RNA on poultry performance. For example, Tipa (2002) reported that broilers fed a diet supplemented with yeast extract had increased BW gain and improved feed conversion ratios as compared to broilers fed a diet without the yeast extract supplementation from 7 to 28 d of age. Owens (2004) also demonstrated that yeast extract supplementation to a broiler diet had a beneficial effect on feed intake and BW gain from 7 to 14 d of age. Similarly, Esteve-Garcia *et al*. (2007) studied the efficacy of a commercial nucleotide preparation (Nucleoforce® Poultry; containing 26.4% of total nucleotide content) in broiler chickens with 3 treatments: 0 (control), 500 and 1000 mg of a nucleotide preparation/kg feed. Birds fed diets supplemented with the nucleotide preparation at 500 mg/kg had higher BW at 21

d of age, as well as, improved feed conversion ratios in the overall experiment period (from 0 to 42 d of age). The authors suggested that supplementation of nucleotides to the diet is very important during the starter period, when digestive organs in chicks develop rapidly. Deng *et al*. (2005) found that the 0.5 and 1.0% *Torula yeast* RNA supplementation as a source of nucleotides did not affect performance of Leghorn-type chickens under normal conditions. Differences among the trials mentioned above could be associated with the available nucleotide content in the different supplement products, the level of nucleotide supplementation to the diets, and no stress/challenge to the birds. In other studies, the supplementation of yeast RNA negatively affected feed intake and BW gain in birds. Kubota and Karasawa (1994) also found that increasing *Torula yeast* RNA (containing 62% total nucleotide content) supplementation from 1% to 5% in 6-d-old Single Comb White Leghorn male chicks for 15 d consistently decreased feed intake and growth rates. Kubota and Karasawa (1997) reported that the inclusion of a toxic level (10%) of a yeast RNA product (contained 86.84% RNA) for 14 d depressed feed intake and growth rates of 14-month-old cockerels. The reasons for the negative effects observed in the aforementioned studies might be due to high single-cell proteins (SCP) in the yeast RNA. Single-cell proteins have been considered as a source of potential protein for animal feed and human diets due to its well-balanced amino acid profile. However, high levels of SCP containing high nucleotide content leads to hyperuricemia and gout development due to increased plasma uric acid levels (Nuget, 1965; Waslien *et al*., 1968; Edozien *et al*., 1970). In birds, the supplementation of SCP to diets decreased broiler growth rate and feed intake when the diet contained more than 100g of SCP/kg (Waldroup *et al*., 1971). Overall, nucleotides supplementation may increase body weight gain and feed intake and improved feed efficiency in

birds. However, incorporation of high amounts of SCP in the form of yeast RNA supplementation could have negative effects.

Laboratory animal studies

A number of studies have been conducted to evaluate the effects of nucleotide supplementation using laboratory animal model systems, such as mice and rats. For example, Yamauchi *et al*. (1998) conducted a study to determine the effect of intraperitoneal administration of a nucleoside-nucleotide mixture on small intestinal morphology, bone marrow cell number, and DNA content in bone marrow in bacterially infected mice fed a protein-deficient and nucleotide-free 20% casein diet. The authors concluded that intraperitoneal administration of nucleoside-nucleotide mixture may regulate bone marrow cell proliferation, DNA content in the intestines, and small intestinal RNA content during periods of protein deficiency in combination with infection. Uauy *et al*., (1990) and Tsujinaka and Toshimasa (1999) also found that dietary nucleotides alone or a nucleoside mixture increased relative weight, protein and DNA content, villous height, and disaccharidase activity in intestine of normal rats or rats with chronic diarrhea. The reason for such effects on bone marrow and intestinal tract may be that both tissues have rapid cell turnover. These rapidly proliferating tissues are not able to produce enough nucleotides exclusively by a *de novo* synthesis, and they preferentially use the salvage pathway. A dietary nucleotide supplementation may be essential to maintain growth and cellular function in theses tissues (Leleiko *et al*., 1995). Dietary nucleotide supplementation also increased Th1 cytokines production. Nagafuchi *et al*. (1997) and Jyonouchi *et al*. (2001) demonstrated that mice fed a free-nucleotide basal diet supplemented with a nucleotide mixture or yeast RNA product increased production of Th1 cytokines, such as

Interleukin-12 (IL-12) and interferon-γ (INF- γ), and thus had improved antigen-specific Th1 immune responses as compared to the mice a the basal diet without nucleotide supplementation. Supplementation of nucleotides or a nucleoside-nucleotide mixture may improve intestinal development through increased villous height, and increased DNA and RNA content and disaccharidase activity in the intestine. Supplementation also increased bone marrow proliferation; IL-12 and INF-γ reflect immune system stimulation. Thus, intestinal development and the immune system appear to be highly dependent on the presence of dietary nucleotides.

GASTROINTESTINAL TRACT

1. Gastrointestinal tract of broilers pre-hatch

The yolk plays a major role in nutrient supply for hatching birds until they can utilize exogenous feed (Uni *et al.,* 2003). Prior to hatch, yolk contents can enter the small intestine of the chicks via the yolk stalk (Geyra *et al.,* 2001). During the 3 days pre hatch, the weight of the small intestine increases at a much greater rate than overall body weight. The embryonic body weight and intestine weight ratio has been shown to increase from 1.4 % at 17 d of incubation period to 3.5% at hatch (Uni *et al*., 2003). The morphology of the small intestine was observed to change dramatically. At 15 d of incubation period the villi of intestinal epithelium were undeveloped and crypts were not observed (Uni *et al.,* 2003). At 17 d of incubation period the embryonic villi and crypts were detected. The embryonic villi are categorized as small and large villi base on their morphological characteristics (Uni *et al.,* 1996). The larger villi were pear-shaped and the smaller villi (approximately 65% of the size of the larger villi) were narrower and rocket-shaped. As the intestinal tract developed, villi height and surface area also increased closer to the hatch. Activity of the brush border enzymes and nutrients transporter

also changed during the last 3 d of incubation period (from 19 to 21 d of incubation) (Uni *et al*., 2003). At 19 d of incubation, the activities of maltase, amino-peptidase, sodium-glucose transporter (SGLT)-1 and ATPase increased rapidly. Pancreatic enzyme activity in the small intestine was also detected prior to hatching. For example, carboxypeptidase A and chymotrypsin rapidly increased from 16 d of embryonic age to 2 d post-hatch, while the activity of pancreatic amylase was detected from 18 d of embryonic age (Marchaim and Kulka, 1967). Lipase activity was also detected prior to hatching (Noy and Sklan, 1998). Thus, the activity of brush border and pancreatic enzymes appear to increase and the morphology characteristics of small intestine of bird develop at the later stages of incubation.

2. Gastrointestinal tract of broilers post-hatch

a. Physical development of the GIT

During the first 7 d post-hatch, relative length, absolute length and weight of the small intestine of broilers dramatically changed (Iji *et al*., 2001a). The weight of small intestine increased faster than the overall body weight (Uni *et al*., 2003). Similarly, Noy *et al*., (2001) reported that the small intestine developed faster than the whole body mass from 6 to 10 d of age. The authors observed that the development of intestinal tract reached its peak prior to 14 d of age and the weight of small intestine relative to body weight peaked at 7 d of age and decreased consistently thereafter. On the other hand, Iji *et al*., (2001a) found that in relation to body weight, gizzard and yolk sac weight decreased with age.

b. Morphological development of the GIT

Villous height and crypt depth

Development of intestinal morphology can be an indication of the health status of the gastrointestinal tract (GIT). Longer villi increase the surface area for absorption in the intestines (Coates *et al*., 1954). Consequently, increased villi height is associated with improved performance due to higher nutrient absorption and utilization (Coates *et al*., 1954; Izat *et al.*, 1989). Bartell and Batal (2007) stated that increased surface area might be also associated with heavier intestinal relative weight. In contrast, shortened villi result in decreased surface area for absorption. Shortened villi in the intestinal tract are also correlated with diarrhea and decreased disease resistance that can lead to decreased performance (Xu *et al*., 2003).

Intestinal crypts are the site of intestinal epithelial cell production. Crypts consist of absorptive cells, goblet cells, and regenerative cells that play a role in the replacement of old intestinal cells (Solis *et al*., 2005). Cells migrate from the crypt to the tip of the villous along the villous surface (Imondi and Bird. 1966). The turnover cycle of the intestinal epithelial cells is approximately 90 to 96 h in birds (Macari, 1998). Deeper crypts are associated with faster turnover of intestinal epithelium, which enhances renewal of the villous as needed in response to normal cell sloughing or in response to cell death due to pathogens or inflammation (Yason *et al*., 1987). Deeper crypts may be associated with disease challenge. Diet may influence the regeneration of intestinal epithelial cells on the villi damaged by disease (Petronini *et al*., 1992; Persia *et al*., 2006).

The first few days after hatching, the intestinal morphology, such as villous and crypt, undergoes rapid change. The villous height in the small intestine increases with age (Uni *et al*., 1999). Consequently, the villous surface area also increases with age to 21 d of age, which was

larger in the duodenum than in either the jejunum or ileum (Iji *et al*., 2001a). Geyra *et al*., (2001) monitored the changes in the morphology of the small intestinal mucosa and enterocyte activity in post-hatch chicks through 12 d of age. At hatch, intestinal epithelium exhibited round or non-polar shapes without distinct villous formation. Within 24 h post-hatch, villi were extended, which was comparable to mature intestinal morphology in all intestinal segments. A further increase in villi length was observed on reaching a plateau of 9 d post-hatch in the duodenum, 6 d post-hatch in the jejunum, and 1 d post-hatch in the ileum. Different patterns of individual and total villi surface area in the duodenum, jejunum and ileum were observed. In the jejunum and ileum the individual villous surface areas increased slowly as compared to those in duodenum after 4 d post-hatch. Whereas, the total segment villous surface area increased similarly in all segments by 3 d post-hatch (Geyra *et al*., 2001). Uni *et al*. (1999) reported that villous height and crypt depth increased rapidly after hatch and reached a plateau after 6 d in the duodenum and 10 d in the jejunum and ileum. Thus, intestinal morphology matures with specific ontogenetic timetables in the different small intestinal segments. Increased villi height or decreased crypt depth is related to improved absorption capacity or less inflammation in the small intestine. Therefore, development of intestinal morphology (villi height and crypt depth) may have a primary function in the processes of nutrient absorption and a relevant role in broiler chicken performance.

Factors that regulate morphology development in GIT

Bacteria infection, heat stress, environmental stress due to high stocking density, and high fiber concentration in animal diets may be factors that affect morphology development such as, villous height and crypt depth in the gastrointestinal tract.

Pathogenic bacteria are one of the main factors affecting the crypt depth and villous height in animals. Bacteria damage a number of the enterocytes and these results in an increase in crypt depth (Deschepper *et al*., 2003). Sakata (1987) found that increased bacteria activity in the GIT resulted in increased villous height in the duodenum. Farthing (2004) reported that bacteria also have an effect on gut development and a resulting reduction in nutrient absorption, immune function, and pathogen resistance at hatch or birth. However, lactobacillus and bifidobacterium may promote gut health by influencing enterocyte turnover and competing with pathogenic bacteria for nutrients (Farthing, 2004).

Heat stress also influences GIT development. Exposure to extremely high temperature is associated with increased intestinal colonization and fecal shedding of pathogens in poultry (Bailey, 1988). Heat stress caused a decrease in integrity of the intestinal epithelium in the rat (Meddings and Swain, 2000). Burkholder *et al*. (2008) reported on the affects of heat stress on intestinal microbial populations and small intestinal morphology in broilers. Birds exposed to 30℃ for 24 h at 44 d of age had decreased ileum crypt depth compared to birds at 23℃ for 24h. Villous height and the villous crypt ratio did not change in responses to increased temperature. The possible reason is that exposure to high temperature may only cause slight changes in epithelial mucus production and reduction of crypt depth (Burkholder *et al*., 2008).

Other factors regulating the development of GIT are high stocking density and the use of various ingredients in animal diets. High stocking density can contribute to change in the small intestinal morphology such as crypt depth and villous height (Puron *et al.*, 1995). The reasons for morphology changes in the small intestine by high stocking density may be due to reduced airflow, high environmental temperature, and increased ammonia, reduced access to feed and water, and increased number of infectious oocysts in the litter (Burkholder *et al.*, 2008; Puron *et*

al., 1995; Lillehoj and Lillehoj, 2000). The use of various ingredients such as corn, wheat, rye, and barley in poultry diets can impact the development of the GIT (Moran, 1985; Carew *et al.,* 1972). Non-starch polysaccharides (NSP) are a major component of some cereal grains. NSP is considered to an anti-nutritional factor and has negative effects on performance and GIT development in birds (Smits and Annison). NSP increased crypt depth and villi height but decreased enterocytes numbers in the small intestine of birds (Iji et al., 2001b).

Challenges, such as pathogenic bacteria, beneficial microflora (lactobacillus and bifidobacterium), heat stress, high stocking density, and high dietary NSP diets, could be factors that affect development of GIT and intestinal morphology. These challenges result in increases in crypt depth, reduction of villi height, decreases in the integrity of the intestinal epithelium and a decrease in nutrient digestibility all leading to a reduction in growth.

C. Functional development of the gastrointestinal tract

Intestinal enzyme activities

Enzymes play a crucial role in the breakdown of nutrients (Murray *et al*., 2003). Digestive enzymes are secreted in and by the gastrointestinal tracts of animals and humans from mainly the stomach, small intestine, and large intestine. The major pancreatic enzymes are amylase, protease and lipase. Amylase breaks down carbohydrates into simple sugars. Protease breaks down proteins into amino acids. Lipase breaks down fats into glycerol and fatty acids (Murray *et al*., 2003). In poultry, enzyme activities are dependent on diet and age. Different dietary carbohydrates will result in changes in the pancreatic enzyme activities (Sell *et al*., 1991; Biviano *et al*., 1993). In general, the total activities of digestive enzymes such as maltase, sucrose, and alkaline phosphates (AP) in the small intestine increased with age (Iji *et al.,* 2001c).

Iji *et al*. (2001c) examined the activities of the main intestinal enzymes in broilers fed a commercial starter diet. The specific activity of maltase, sucrase, amonopeptidase N (APN), and alkaline phosphatase (AP) per unit membrane protein at all intestinal sites decreased with age in all intestinal regions. The variation between the intestinal regions was dependent on age and individual enzymes. Uni *et al*. (1999) found that mucosal disaccharidase (maltase and sucrose) activity per gram of tissue in each segment peaked at 2 d post-hatch, then decreased to the lowest point at 5 to 6 d of age, and then increased until it peaked at 12 d of age. Activity of alkaline phosphates (ALP) peaked at 2 d of age and then reached a plateau until 12 d of ages in the duodenum and ileum. Total enzyme activity (μmol product/membrane) increased with age (between hatch and 21 d of age) in all intestinal regions. Uni *et al*., (1999) also found that the calculated specific activity of maltase, sucrase, and alkaline phosphatase significantly increased with age until 12 d of age. The increase in enzyme activity with age is mainly due to an increase in the entire weight and length of the intestinal tissues and increased villus height (Iji *et al*., 2001c).

COCCIDIOSIS

Coccidiosis is an intestinal infection caused by intracellular protozoan parasites that belong to the genus *Eimeria* (McDougald, 1997). *Coccidiosis* is one of the most troublesome diseases in the poultry industry economically. The amount spent annually in the US on anticoccidial drugs has been estimated to be 127 million dollars (Chapman, 2009). The major clinical symptoms of *Coccidiosis* are intestinal hemorrhage, malabsorption, diarrhea, and reduced body weight gain due to the disruption of intestinal function (Lillehoj and Lillehoj, 2000). *Coccidiosis* also stimulates the broilers immune system; increasing the level of interferon

gamma (INF-γ) and production of nitric oxide (NO), which can be used as an indicator of epithelial proliferation (Lillehoj and Lillehoj, 2000).

Seven *Eimeria (E)* species have been found to infected chickens; *E. acervulina, E. maxima, E. tenella, E. brunette, E. necatrix, E. mitis, and E. praecox.* Each species has its own characteristics, location of infection, pathogenicity, and immunogenicity (Rose and Long, 1980). Three species; *E. acervulina, E. maxima, and E. tenella* have been identified as the main cause of *Coccidiosis* infections in commercial poultry (McDougal *et al*., 1997).

1. Life cycle of *Eimeria spp*.

The life cycle of *Eimeria species* involves three stages of development: sporogony, merogony, and gametogony. The first stage, sporogony takes place outside of the host. The infected chickens shed oocysts in the external environment. The oocysts excreted in this way have to sporulate in order to become infectious. The sporulated oocysts contain four sporocysts, each containing two sporozoites. The birds ingest these infective sporozoites orally. Once ingested, sporocysts are ground mechanically in the gizzard allowing sprozoites to be released. In the duodenum, the external wall of the sporocyst is dissolved by the action of pancreatic enzymes, mainly trypsin, and bile salts. Trypsin acts to release sporozoites into the intestine and bile salts enhance sporozoites activity and motility (Current *et al*., 1990). Depending on the species of parasite, the mobile sprozoites move to a specific area of infection and actively invade epithelial cells in the small intestinal villi, crypt epithelial cells, and lamina propria. Once inside the host cell, sprozoites start the second stage of development called merogony in which nuclear division is followed by cytoplasmic differentiation, resulting in the formation of meronts. These meronts may contain a number of merozoites. When the meronts are mature, these merozoites in

the meronts can burst and penetrate other epithelial cells and repeat this process of asexual multiplication. The gametogeny follows the last merogonic cycle. The last generation merozoites invade the host cells and develop into either male (microgamonts) or female (macrogamonts) forms. The microgamonts undergo nuclear divisions and produce many microgametes in the host cell. On the other hand, macrogamete undergoes maturation without cell division and thus, engenders only one macrogamete. However, the macrogametes grow in size while they proliferates cellular organelles within the host cell. The microgametes invade cells including macrogametes and the macrogamete is fertilized by the penetrating microgamete which results in the formation of a zygote. After fertilization, the oocysts mature as they are released from the host cell and then discharged in the feces. The sporulation process then starts a new infective cycle (Current *et al*. 1990).

2. Effects of *Coccidial* infection

Eimeria acervulina infection

Eimeria (E) acervulina is a protozoan parasite and lives mainly in the duodenum of the chicken. *E. acervulina* leads to many morphological changes in intestinal epithelium; mucosal soughing, truncated and thickened villi, increased crypt depth, increased epithelial cell turnover, decreased total mucosal dry weight, and increased relative length and weight of intestine (Witlock and Ruff, 1977; Allen, 1984 and 1987). Allen (1984) studied the effects of *E. acervulina* infections of 2×10^6 sporulated oocysts/bird on the length and fresh weight of small intestine in chickens. This *E. acervulina* infection significantly increased the length of duodenum and jejunum at 7 and 8 d post-infection. The chickens infected with *E. acervulina* had longer ileums at 4 to 8 d post-infection as compared to the birds that were not infected. However, the *E.*

acervulina infection at 2×10^6 sporulated oocysts/bird did not affect fresh weight of the duodenum but did increase the fresh weight of the jejunum and ileum. Another study conducted by Allen (1987) evaluated the effect of a *E. acervulina* infection on total mucosal dry weight of the small intestines of chickens. The author reported that chicks infected with 2×10^6 sporulated oocysts/bird had significantly lower total mucosal dry weight which is an indicator of mucosal hypertrophy or hyperplasia (Fernando and McCraw, 1973) of the duodenum as compared to the birds non-infected at 3, 5, and 7 d post-infection (PI). In addition, *E. acervulina* infection significantly decreased total dry mucosal dry weight of jejunum compared to non-infected birds at 5 and 7 d PI. In contrast, total dry weights of the ileum were increased due to the infection of *E. acervulina*. Allen (1984) suggested that although *E. acervulina* infects primarily the duodenum of chickens, the entire small intestine might be affected. In addition, *E. acervulina* infection at 1×10^6 sporulated oocysts/bird negatively impacted body weight gain, feed efficiency, nutrient absorption, and lesion scores (Conway *et al*., 1993). The infection of *E. acervulina* at 2×10^6 oocysts/bird also significantly decreased the levels of plasma carotenoid (an indicator of nutrient malabsorption), decreased mucosal alkaline phosphatase and sucrase activities in duodenum and jejunum (an index of morphological maturation and differentiation of mucosal cells) (Allen, 1987; Ruff and Edgar, 1982; Falchuck *et al*., 1974).

Eimeria maxima infection

Eimeria (E) maxima mainly affect the jejunum, which is the middle portion of the small intestine. *E. maxima* are the most highly immunogenic coccidian species in chickens (Ruff and Reid, 1977). *E. maxima* infection increases mortality, morbidity decreasing body weight gain and feed efficiency of birds during the acute phase of the infection (5 to 7 d post-infection). *E.*

maxima also damages intestinal epithelium, increasing the sloughing of the mucosa that leads to nutrient malabsorption and reduced body weight gain of birds (Stephens *et al*., 1967; Ruff, 1978). In addition, *E. maxima* infection reduced plasma levels of carotenoid (Allen, 1984) and increased the level of interferon gamma (INF-γ), which indicated increased inflammation (Chapman *et al*., 1982; Laurent *et al*., 2001). Thus, *E. maxima* infection causes lesions mainly in the jejunum that lead to substantial nutrient malabsorption, increased inflammation and reduced body weight gains and feed efficiency.

Eimeria tenella infection

Eimeria (E) tenella infection leads to lesions in the ceca and large intestine of chickens that result in reduced performance of birds during the acute phase of a *Coccidiosis* infection (Witlock and Ruff, 1977). This parasite infection can cause increased length and weight of intestine, inflammation, mucosal disruption, and hemorrhage in intestinal epithelium, as well as mortality of birds (Allen, 1997). Sharma *et al.*, (1973) reported that a single dose infection (1×10^5 oocysts of *E. tenella*) reduced daily gain, feed intake, and feed conversion ratios of birds during the first 8 d post-infection. The authors mentioned that the reduction in weight gain was more severe in chicks infected with *E. tenella* as compared to the chicks infected with *E. acervulina*. Oikawa *et al*. (1971) also suggested that *E. tenella* has a greater pathogenicity than *E. acervulina* in birds. *E. tenella* causes lesions in the ceca and large intestine and leads to reduced performance and increased intestinal weight and length of birds during the acute phase of *Coccidiosis*.

Live oocyst vaccination

The use of a live oocyst vaccination is a practical alternative to anticoccidial drugs to control *Coccidiosis* infection in poultry by enhancing natural immunity of chickens (Chapman *et al.,* 2002). The commercial C*occidia* vaccines contain the 3 most prevalent *Coccidia* species in chickens; *E. acervulina, E. maxima*, and *E. tenella* (Lillehoj and Lillehoj, 2000). In general, this vaccination leads to a temporal growth reduction after inoculation, which is related to increased incidence of secondary enteritis (Chapman *et al*., 2002). However, live oocyst vaccination results in enhanced immunity, increased final body weight gain, improved feed conversion ratios, reduced lesion scores, and reduced mortality rates (Danforth, 1998; Crouch *et al.*, 2003; Williams, 2003).

The leading commercial vaccines used in the poultry industry today are Coccivac®, Immucox®, Livacox®, and Paracox®. Coccivac® and Immucox® are non-attenuated vaccines because they use *Coccidian* species isolated from commercial poultry production facilities. On the other hands, Livacox® and Paracox® are attenuated vaccines, which utilize egg-adapted or precocious lines originated *Coccidia* species (Danforth, 1998). Several reports have evaluated the use of live oocyst vaccinations for control of *Coccidiosis* in broilers. Williams (1994) studied the effect of an attenuated vaccine (Paracox®) on broiler performance and reported that during the grow-out period (54 d of age), vaccinated broilers had heavier body weights, had improved feed conversion ratios, and reduced mortality as compared to the unvaccinated broilers. Crouch *et al.* (2003) also investigated the effect of an attenuated vaccine, Paracox®-5, on broiler performance and lesion scores. Broilers were vaccinated with Paracox®-5 on 0 d of age and then challenged with each species: *E. acervulina, E. maxima, E. mitis*, or *E. tenella* at 28 d of age. The vaccinated birds had increased body weight gains and improved feed conversion ratios as

compared to non-vaccinated birds. Lesion scores were also significantly lower in vaccinated birds as compared with non-vaccinated birds. Yhe use of anticoccidial vaccines in broilers has been shown safe and effective at protecting broilers from a *Coccidiosis* infection.

3. Effects of dietary supplementation during a *Coccidiosis* infection

Studies have been conducted to test the effects of betaine and omega-3 fatty acids supplementation on performance, nutrient absorption, mortality, lesions scores, and immune system of broilers challenged with *Coccidiosis*. Betaine is a bipolar electrolyte that is used as a feed additive in birds. It acts as an osmoprotectant (Petronini *et al*., 1992). *Coccidiosis* infections lead to diarrhea and dehydration in birds that result in osmotic stress (Allen *et al*., 1998). Thus, the supplementation of betaine to the diets may have beneficial effects on performance of birds infected with *Coccidiosis* (Petronini *et al*., 1992). Numbers of broiler trials have examined the effects of betaine and its combination with antibiotics, such as salinomycin or monensin, on performance, lesion scores, and mortality of birds infected with *Coccidiosis*. Matthews *et al*. (1997) reported that *E. accervulina* infected birds fed diets supplemented with 0.1% betaine had heavier body weight and better feed:gain ratios than the birds fed the control diet. Coccidiosis infected birds fed a diet supplemented with 0.5% betaine had improved feed efficiency as compared to the birds fed the control diet. Klasing *et al*. (2002) also examined the effects of dietary betaine on performance, intestinal osmolarity, leukocytes levels and intestinal morphology in broiler infected with *E. acervulina*. The addition of 0.1% betaine in the broiler diets reduced the number of intraepithelial leukocytes and osmolarity of the duodenum caused by a *Coccidiosi*s infection. The 0.1% betaine addition also ameliorated the negative effect of an *E. acervulina* infection on villous height.

Omega-3 fatty acids may also reverse the negative effects of *Coccidiosis* on performance of broilers. A few studies have been conducted to determine the effect of *Eicosapentaenoic acid* (EPA) and *docosahezaenoci acid* (DHA) on broiler performance and lesion scores during *E. species* infection in birds. The addition of 10 % fish meal, which is high in ω-3 fatty acids, to a broiler diet improved performance during an *E. acervulina* infection (Persia *et al*., 2006). In addition, dietary supplements of flaxseed that is also rich in ω-3, was found to be effective to reduce lesion scores due to an *E. tenella* infection in birds. However, it did not reverse the negative effects of the *E. tenella* infection on performance parameters such as body weight gain (Allen *et al*., 1997). Overall, the negative effects of *Coccidiosis* infection are ameliorated by the diet supplementation of betaine or ingredients high in omega-3 fatty acids.

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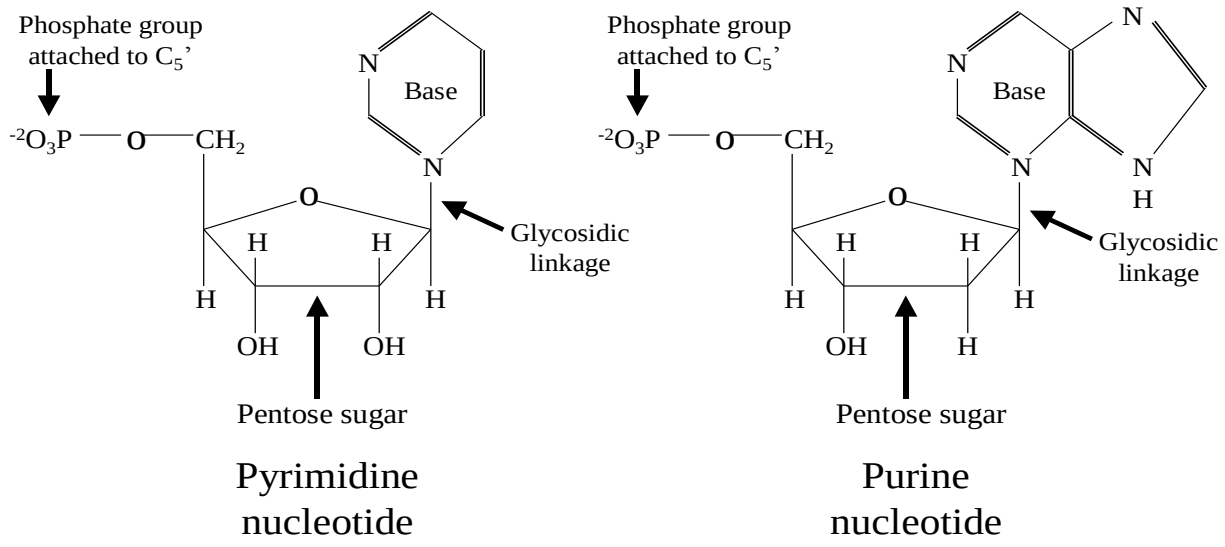
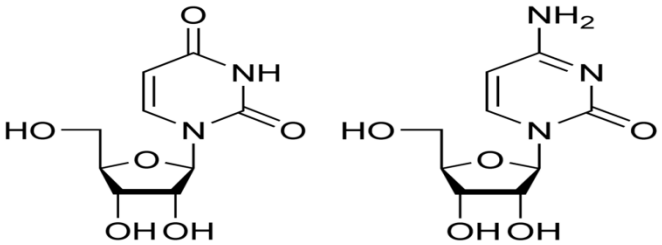
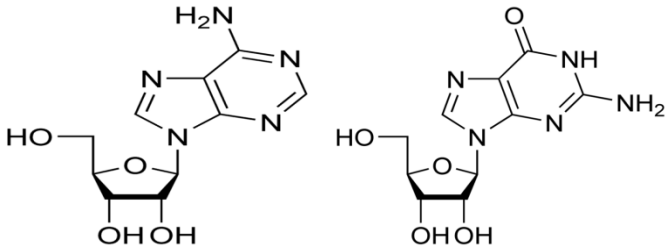


Figure 1.1. Generalized structure of a nucleotide (Adapted from Stein and Mateo, 2005).

C = carbon atom, H = hydrogen atom, O = oxygen atom, and N = nitrogen atom



Uridine Cytidine



Adenisine Guanosine

Figure 1.2. Pyrimidine (uridine and cytidine) and purine (adenosine and guanosine) bases of nucleosides.

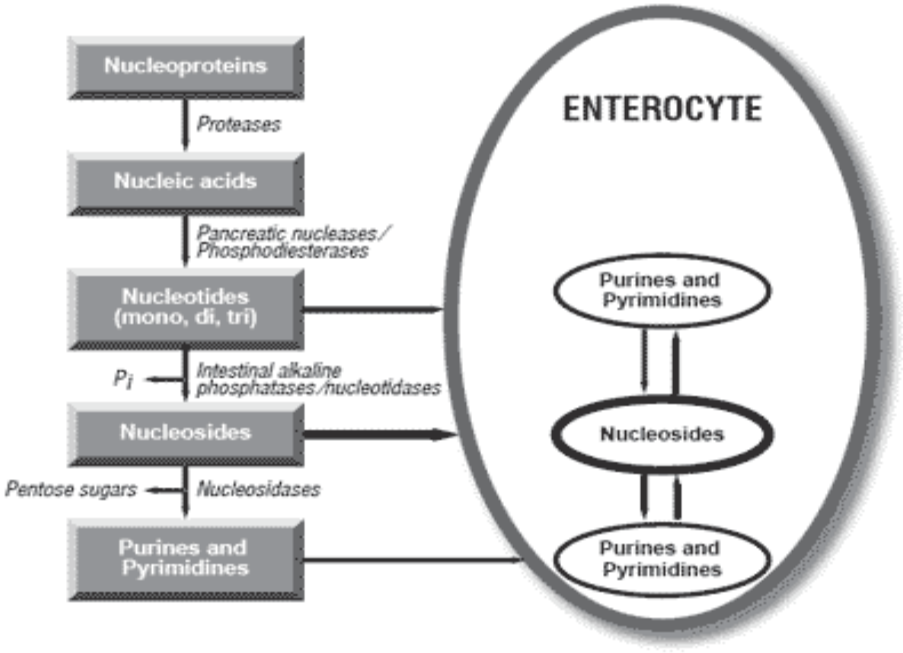


Figure 1.3. Absorption and metabolism of nucleic acids and their related products; Adapted from Quan and Uauy (1991).

Table 1.1. Nucleotide concentration[1] (mg/kg) in some commonly used feed ingredients (as-is basis)

Ingredients	5'AMP[2]	5'CMP[2]	5'GMP[2]	5'IMP[2]	5'UMP[2]	Total nucleotides
			---------- mg/kg ----------			
Non-fat dried milk	0	65	0	195	106	366
Dried whey	19	270	0	4	1	294
Whey protein concentrate	0	34	0	159	89	282
Corn	2	3	3	1	0	9
Soybean meal, 44%	8	16	3	2	9	38
Casein	0	1	0	0	0	1
Barley	1	2	1	1	0	5
Fish meal	11	26	2	35	1	75
Naked oats	3	3	3	1	1	11
Plasma protein, spray dried	2	2	2	1	0	7
Red blood cells, spray dried	44	0	3	6	2	55
Soy protein concentrate	1	0	2	1	0	4

[1] Adapted from Mateo (2005).

[2] 5'AMP = Adenosine 5'monophosphate; 5'CMP = Cytidine 5'monophosphate; 5'GMP = Guanosine 5'monophosphate; 5'IMP = Inosine 5'monophosphate; 5'UMP = Uridine 5'monophosphate.

Table 1.2. Purine, RNA, and protein content[1] of selected foods.

Items	Adenine	Guanine	Hypo-xanthine	Xanthine	Total purines	RNA	Protein
			----------	%	----------		
Organ meats							
Beef liver	0.62	0.74	0.61	0.00	1.97	2.68	20
Beef kidney	0.42	0.47	0.63	0.61	2.13	1.34	18
Beef heart	0.15	0.16	0.38	1.02	1.71	0.49	19
Beef brain	0.12	0.12	0.26	1.12	1.62	0.61	11
Pork liver	0.59	0.77	0.71	0.82	2.89	2.59	22
Chicken liver	0.72	0.78	0.71	0.22	2.43	4.02	20
Chicken heart	0.32	0.41	0.12	1.38	2.23	1.87	18
Lamb liver	0.32	0.43	0.54	0.18	1.47	0.88	22
Lamb heart	0.30	0.23	0.20	0.98	1.71	0.50	19
Fresh sea-foods							
Anchovies	0.08	1.85	0.06	2.12	4.11	3.41	20
Salmon	0.26	0.80	0.11	1.33	2.50	2.89	23
Sardines	0.06	1.18	0.06	2.15	3.45	3.43	23
Squid	0.18	0.15	0.24	0.78	1.35	1.00	15
Dried legumes							
Cranberry bean	0.21	0.19	0.23	0.12	0.75	2.48	17
Lentils	1.04	0.82	0.20	0.16	2.22	4.84	28
Blackeye peas	0.77	0.80	0.32	0.41	2.30	3.06	22
Baby lima bean	0.46	0.39	0.25	0.34	1.44	1.90	19

[1] Adapted from Clifford and Story (1976).

Table 1.3. Purine and Pyrimidine bases content[1] of some important feedstuffs

Ingredient	Nucleotide bases, %					Total bases
	Cytosine	Uracil	Guanine	Thymine	Adenine	
Complete fish meal	0.12	0.12	0.94	0.05	0.17	1.41
Press cake fish meal	0.08	0.08	0.13	0.02	0.10	0.42
Fish soluble	0.11	0.12	2.33	0.08	0.19	2.83
Yeast	0.18	0.19	0.25	0.02	0.28	0.90
Yeast extract	0.14	0.73	0.62	0.03	0.73	2.30
Single cell protein	0.10	0.55	0.88	0.37	0.17	2.07

[1] Adapted from Devresse, B. (2000).

CHAPTER 2

EFFECT OF DIETARY NUCLEOTIDE SUPPLEMENTATION ON PERFORMANCE AND DEVELOPMENT OF THE GASTROINTESTINAL TRACT OF BROILERS[1]

[1] Jung, B. and A. B. Batal. Submitted to *British Poultry Science*.

ABSTRACT

Two experiments were conducted to determine the effects of dietary nucleotide supplementation on broiler performance and on the physical and morphological development of the gastrointestinal tract. In experiment 1, one hundred and eighty 1-d-old male chicks were placed in battery brooders in pens of ten chicks each. Pens were randomly assigned to one of the three dietary treatments; a corn-soybean meal based diet supplemented with 0, 0.25, and 0.50% *Torula yeast* RNA (as a source of nucleotides) such that each treatment was administreted to six replicate pens. Diets were fed for 16 d of age. Supplementing the diets with *Torula yeast* RNA did not affect broiler performance or relative intestinal tract weight and length of broilers. In experiment 2, one thousand three hundred and forty four 1-d-old male chicks were placed in floor pens and reared on wood shavings which had been previously used for two flocks of broilers. Chicks were randomly assigned to one of four dietary treatments (0, 0.25% *Torula yeast* RNA, 2% and 6% Nupro®) for the starter period (0 to 14 d of age). Treatments were fed to six replicate pens containing fifty six chicks each. All the birds were fed the same standard grower diet with no supplementation of nucleotides from 15 to 32 d of age. From 0 to 14 d of age, broilers fed the diets supplemented with 0.25% *Torula yeast* RNA and 2 and 6% Nupro® were significantly heavier and had improved feed conversion (feed:gain) ratios as compared to the birds fed the control diet. Supplementing the starter diet only with 2% Nupro® significantly improved BW gain as compared to the control diet over the entire experiment (0 to 32 d of age). Broilers fed diets supplemented with 2 and 6% Nupro® from 0 to 14 d of age had better feed conversion (feed:gain) ratios over the entire experiment (0 to 32 d of age) as compared to the birds fed the control diet even though the birds were only fed the diets supplemented with Nupro® from 0 to 14 d of age. The broilers fed the diets supplemented with 0.25% *Torula yeast*

RNA and 2% Nupro® had higher villous height and an improved villous height to crypt depth ratio as compared the birds fed the control or 6% Nupro® diet at 14 d of age. It is generally assumed that nucleotides are not an essential nutrient, thus there is no need to supplement the diets of broilers reared under normal conditions. However, dietary nucleotide supplementation may be important to maintain maximum growth performance when birds are exposed to environmental stress conditions, such as previously used litter.

(**Key words:** Nucleotides, broilers, performance, villous height, stress conditions)

INTRODUCTION

Nucleotides are the basic units of nucleic acids (DNA and RNA). The molecular structure of nucleotides is based on the combination of a sugar, ribose or deoxyribose, a nitrogen base, such as a purine or a pyrimidine, and one or more phosphate groups (Voet and Voet, 2004). Nucleotides play key roles in numerous biological processes; they are incorporated in nucleic acid precursors, components of coenzymes, sources of cellular energy, involved in the synthesis of DNA and RNA, and mediate hormonal action (Carver, 1999). Nucleotides are synthesized within cells by a *de novo* pathway from amino acid precursors such as glutamine, formate, glycine and aspartic acid. They are also recycled through a salvage pathway. In the salvage pathway, during degradation of nucleic acids, nucleosides and/or bases are re-utilized for biosynthesis of nucleotides (Carver and Walker, 1995). Over the past several years, studies have indicated that both mechanisms may not provide enough nucleotides to maintain normal biological functions under certain conditions such as rapid growth, reproduction, malnutrition, recovery from injuries, environmental changes, and disease challenges (Yu, 1998; Carver, 1999). Thus, nucleotides have been considered a semi-essential nutrient in humans and animals.

A small number of studies have indicated that providing supplemental nucleotides in animal diets has beneficial effects on performance and intestinal morphology development. Esteve-garci *et al*. (2007) found that the supplementation of 0.05% of a commercial nucleotide product (Nuceloforce® ; containing 26.4% of balanced free nucleotides) to broiler diets increased body weight (BW) gain and improved feed conversion ratios when compared to birds fed the control diet.. Uauy *et al*. (1990) reported that rats fed diets with nucleosides had higher villous height in the duodenum as compared to the rats fed a nucleoside-free diet. Maribo (2003) reported that supplementing the diets of pigs infected with E. coli with a 4.0% yeast extract

product (Nupro® ; contained 7% of free nucleotides) increased BW gain and improved feed conversion ratios as compared to the control diet. Cruickshank (2002) and Maribo (2003) suggested that stress conditions, such as environmental changes or challenge and infection stress is required to see positive effects on performance due to the addition of yeast products containing high nucleotides in broilers or pigs.

The objective these studies was to determine the effect of supplementing diets with nucleotide products (*Torula yeast* RNA as a source of nucleotide supplementation and a commercial nucleotide product, Nupro®) on performance and physical and morphological development of the gastrointestinal tract in broilers under normal and stress conditions (increasing stocking density combined with previously used litter).

MATERIALS AND METHODS

All experimental protocols using animals were approved by the Animal Care and Use Committee at the University of Georgia.

Experiment 1

Birds and treatments

Three mash corn-soybean meal based experimental diets were formulated to be isocaloric and isonitrogenous at 3,050 kcal ME/kg and 21.5 % CP, respectively. The three experimental diets contained *Torula yeast* RNA at 0, 0.25 and 0.50%. The total calculated nucleotide level in the experimental diets was 0.38, 0.53, and 0.66%, respectively (Table 2.1). One hundred and eighty 1-d-old male Cobb-500 chicks were housed in an environmentally controlled building and placed into thermostatically controlled starter batteries (Petersime Incubator Co., Gettysburg,

OH) with trough type feeders and waterers. All birds had *ad libitum* access to the feed and water with a 24 h lighting schedule. Chicks were randomly assigned to one of the three aforementioned experimental diets with six replicate pens containing ten chicks each. Chicks were fed the treatment diets from 0 to 16 d of age. Body weight (BW) gain and feed intake were measured on 3, 8, and 16 d of age and feed conversion (feed:gain) ratios were calculated at each time point. When mortality was observed, feed intake was adjusted based on bird days on feed.

Analysis of the physical development of the intestines

Two chicks from each replicate, a total of twelve chicks from each treatment, were randomly selected and euthanized on 3, 8, and 16 d of age for intestinal weight and length measurements as described by Miles *et al*. (2006). Chicks were weighed on an electronic scale. The abdominal cavity was opened by a midline incision to remove the small intestine, which was divided into three segments: duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction). Contents were removed from the intestinal segments by flushing with a saline solution and the relative weight (g/100g of BW) and the length (cm/100g of BW) of the emptied small intestine were determined.

Experiment 2

Birds and treatments

Four experimental corn-soybean meal based diets were formulated to contain 0 or 0.25% *Torula yeast* RNA and 2 or 6% of a commercial nucleotide product (Nupro®). Nutritional

composition of the commercial nucleotide product was analyzed by the manufacturer[1] (analysis summarized in Table 2.2). The total calculated nucleotide level in the diets for the starter period (from 0 to 14 d of age) is shown in Table 2.3. All the birds were fed a common grower diet with no supplementation of nucleotides for the grower period from 15 to 32 d of age. The experimental diets for the starter period were formulated on a digestible amino acid basis (Baker, 1997) and were formulated to be isonitrogenous (21% CP) and isocaloric (3,080 kcal ME/kg) (Table 2.3). Calculated digestible lysine, total sulfur amino acid (TSAA) and threonine content in the diets for the starter period were 2% lower than Baker's (1997) recommendations, whereas the calculated digestible content of all the other amino acids exceed Baker's (1997) recommendations for the grower period (Table 2.4). One thousand three hundred and forty four, 1-d-old male Cobb-500 chicks were housed in an environmentally controlled building and placed in floor pens equipped with hanging tube feeders, a Ziggity nipple drinker (Ziggity Systems Inc., Milford, IN). Birds were reared on wood shavings on which 2 flocks of broilers had been grown previously. Chicks in each of the four treatments were planced in six replicate pens containing fifty-six chicks each. The pen size was a 3.11 m × 1.22 m with a stocking density 14.8 birds / m^2. Chicks were maintained on a 24 h lighting schedule for the first 3 d and thereafter a 23 h lighting schedule (1h dark). Chicks had *ad libitum* access to feed and water from 0 to 32 d of age. Body weight (BW) gain and feed intake were measured on 14, 24, and 32 d of age and feed conversion (feed:gain) ratios were calculated. At the occurrence of mortality, feed intake was adjusted based on bird days on feed.

[1]All-tech® , Nicholasville, KY, 40356.

Analysis of the intestinal morphology

One broiler chick from each pen (total 24 birds) was euthanized and jejunum samples (from entry of the pancreatic ducts to yolk stalk) were taken at 14 d of age for intestinal morphology analysis. After emptying and flushing the jejunum with saline solution, approximately 5cm of the middle portion of the jejunum was excised with a surgical blade and fixed in 10% formalin. Three cross sections of the formalin-preserved segments from each jejuna sample were then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures (Uni *et al*., 1995). A total of three intact well-oriented villi were selected in three replicates from each jejuna cross section (9 measurements for each jejuna sample with 54 measurements per treatment). Villous height was measured from the tip of the villous to the bottom of the villous, and crypt depth was measured from the villous bottom to the crypt. Villous height and crypt depth ratio were calculated. Morphological indices were determined using computer-aided light microscopy (10× magnification of the objective lens and 1× magnification of the adapter lens) with image software analysis (Image-Pro Plus Version 3.0, Media Cybernetics, Silver Spring, MD 20910).

Statistical Analysis

Data from all experiments were analyzed using the ANOVA procedure of SAS® software (SAS Institute, 2005) for completely randomized designs. Statistical significance of differences among treatments was assessed using the Duncan's new multiple range test. A probability level of $P < 0.05$ was used to determine statistical significance of differences among the dietary treatments.

RESULTS AND DISCUSSION

There were no differences in broiler performance and relative weight and length of the small intestine due to the supplementation of 0.25 or 0.50% *Torula yeast* RNA under normal environmental conditions at any periods measured in experiment (exp.) 1 (Table 2.5 and Table 2.6). It appears that the nucleotide content in the control diet was sufficient for optimal growth and physical development of the small intestine when the birds were raised under normal conditions. Cruickshank (2002) and Maribo (2003) suggested that any effect on growth performance due to nucleotide supplementation may only be observed under stress conditions, such as environmental changes or challenge and infection. Experiment (exp.) 2 was conducted to determine the effect of supplementing *Torula yeast* RNA and a commercial nucleotide product (Nupro®) as a source of nucleotides on performance and intestinal morphological development when birds were exposed to environmental stress conditions; increasing stocking density combined with previously used litter.

Body weight (BW) gain, feed intake, and feed conversion (feed:gain) ratios results from exp. 2 are summarized in Table 2.7. Results from this experiment demonstrated that there was no difference on feed intake among treatments. Birds fed diets supplemented with 0.25% *Torula yeast* RNA, 2 and 6% Nupro® gained significantly more weight and had improved feed:gain ratios as compared to birds fed the control diet during the starter period (0 to 14 d of age). However, when birds were fed a common grower diet with no supplementation of nucleotides during the grower period (15 to 32 d of age), no difference in BW gain during the grower period was observed among the all treatments. Overall (0 to 32 d of age) the supplementation of 2% Nupro® to the control diet significantly increased BW gain as compared to the control diet, even through the birds were only fed diets supplemented with 2% Nupro® during the starter period (0

to 14 d of age). Similarly, Rutz *et al.* (2006) found that broilers fed a diet containing 2% yeast extract (Nupro® : contained 7% of total nucleotide content) had significantly better BW gains from 0 to 14 d of age as compared to birds fed an un-supplemented yeast extract diet even though the birds were only fed the yeast extract diets until 7 d of age. From 0 to 32 d of age the feed:gain ratios of birds fed the diets supplemented with 2 or 6% Nupro® were significantly better as compared to the birds fed the control diet. Esteve-garcia *et al.* (2007) found that supplementing broiler diets with 0.05% nucleotide preparation (Nucleoforce® Poultry: containing 26.4% of balanced free nucleotides) resulted in higher BW gains and improved feed:gain ratios from 0 to 42 d of age. When pigs were infected with *E. coli*, the supplementation of 4.0% yeast extract (Nupro® ; containing 7% of free nucleotides) significantly increased BW gain and improved feed conversion (feed:gain) ratios as compared to the pigs fed the control diet without yeast extract supplementation (Maribo, 2003). A few studies have not found any benefits due to the addition of nucleotide or yeast extract products to poultry and swine diets. Deng *et al*. (2005) reported that the supplementation of 0.5 or 0.1% *Torula yeast* RNA (containing 62% total nucleotide content) from 0 to 4 weeks of age did not affect BW gain and feed:gain ratios of White Leghorn chickens (0 to 12 weeks of age). Andres-Elias *et al*. (2007) and Martinez-Puig *et al*. (2007) also reported that there were no significant effects on performance due to the addition of 0.10, 0.15, or 0.20% nucleotide rich yeast extract containing 25% free nucleotides to the diets of weaned pigs. Sufficient amounts of nucleotides provided from the basal diet might explain the lack of influence on performance in growing birds or weaned pigs. Differences between effects and no effects due to the supplementation of nucleotides observed from the studies mentioned above could be associated with the content of available nucleotides of the different supplements and yeast extract products. Also, the nucleotides level in the basal diet, the level of nucleotide

supplementation to the diets, no stress conditions, health status of the birds, and environments may be important factors that may explain the difference in previous experiments.

The jejuna morphology results of birds fed diets supplemented with *Torula yeast* RNA or Nupro® to 14 d of age are summarized in Table 2.8. Birds fed diets supplemented with 0.25% *Torula yeast* RNA and 2% Nupro® had significantly ($P < 0.05$) higher villous height and villous height to crypt depth ratio as compared to the birds fed the control and 6% Nupro® diet. However, there was no effect on crypt depth due to the addition of *Torula yeast* RNA and Nupro® to the diets. Similarly, some swine and rat studies have reported that the supplementation of dietary nucleotides increased villous height. Martinez-Puig *et al*. (2007) reported that early weaned pigs fed diets supplemented with 0.1% or 0.2% commercial nucleotides-rich yeast extract products (containing 25% free nucleotides) had higher villous height as compared to pigs fed diets with no supplementation of the yeast extract product. Domeneghini *et al.* (2004) also demonstrated that the supplementation with dietary nucleotides prevented villous atrophy in post-weaning pigs. Uauy *et al*. (1990) reported that rats fed nucleoside-supplemented diets had increased villous height as compared to rats fed a control diet. Rapid development of mucosal villous allows chicks to utilize nutrients more efficiently in their early life and improves growth performance (Bartell and Batal, 2007). Lilja (1983) noted that avian species have a high growth rate capacity that was related to a rapid early development of the digestive tract. Thus, improved growth performance could be achieved by rapid digestive tract development in growing birds. Izat *et al.*, (1989) also mentioned that increased villous height has been correlated to increased performance due to improved nutrient absorption. The raised villous height that was observed might suggest that the birds fed diets supplemented with 0.25% *Torula yeast* RNA and 2% Nupro® had greater nutrient absorption and utilization because increases in villous height

resulted in more surface area for nutrient utilization. The increased surface area might also explain the significantly increased BW gain and improved feed efficiency from 0 to 14 d of age that were observed due to 0.25% *Torula yeast* RNA and 2% Nupro® supplementation.

In conclusion, dietary nucleotide supplementation to diets did not affect physical development in gastrointestinal tract and performance when birds are not exposed to obvious stress conditions. Thus, nucleotides are not considered an essential nutrient for use in poultry dietary programs. However, based on the results of exp. 2, dietary nucleotide supplementation may be an important factor for birds to maintain maximum performance under certain stress conditions such as previously used litter.

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Table 2.1. Composition of the dietary treatments (as-fed basis) from 0 to 16 d of age.

Experiment 1.

Ingredients, %	Control	0.25% *Torula yeast* RNA[1]	0.50% *Torula yeast* RNA[1]
Corn	59.0	59.0	59.0
Soybean meal	34.9	34.9	34.9
Poultry fat	2.15	2.30	2.45
Torula yeast RNA[1]	-	0.25	0.50
Limestone	1.37	1.37	1.37
Dicalcium phosphate	1.73	1.73	1.73
Salt	0.35	0.35	0.35
Vitamin mix[2]	0.25	0.25	0.25
Mineral mix[3]	0.08	0.08	0.08
DL-Methionine	0.20	0.20	0.20
L-Lysine-HCl (79%)	0.06	0.06	0.06
Total	100	100	100
Contents by calculation			
ME_N, kcal/kg	3,050	3,050	3,050
Crude protein, %	21.5	21.5	21.5
Calcium, %	1.00	1.00	1.00
Available phosphorus, %	0.45	0.45	0.45
Dig. Lysine (1.12)[4], %	1.23	1.23	1.23
Dig. TSAA[5] (0.81)[4], %	0.84	0.84	0.84
Total nucleotides by calculation[6]	0.38	0.53	0.66

[1]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[2]Vitamin mix provided the following (per kg of diet) : Thiamin mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12mg; vitamin B_{12}(cobalamin), 12.0µg; pyridoxine HCL, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5µg; trans-retinyl acetate, 1,892µg; all-rac α tocopheryl acetate, 11mg; ethozyquin, 125mg.

[3]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; idodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.

[4]Digestible amino acid recommendations for starter broiler chickens (Baker, 1997).

[5]TSAA = Methionine +Cystine.

[6]Calculations based on the analysis from Chemoforma Ltd, Rheinstrasse 28-32 Augst BL, Switzerland.

Table 2.2. The nutritional composition[1] of a commercial nucleotide product, Nupro® (Dry matter basis).

AME_N, kcal/kg	2,954	Macro minerals, %	
Crude protein, %	50.02	Calcium	0.96
Crude fat, %	0.45	Phosphorus	1.63
NFE[2], %	33.01	Sulfur	0.50
Total ash, %	8.84	Sodium	0.11
		Potassium	2.02
Amino acids, %		Magnesium	0.47
Lysine	3.40		
Alanine	3.13	Trace minerals, ppm	
TSAA[3]	1.26	Iron	561
Arginine	2.14	Copper	32
Aspartic	4.45	Zinc	255
Glutamic Acid	6.76	Manganese	69
Glycine	2.21	Chloride	4,300
Histidine	1.13		
Isoleucine	1.96	Vitamins, ppm	
Leucine	3.00	Biotin	0.42
Phenylalanine	1.89	Inositol	3,359
Proline	1.53		
Serine	2.10		
Threonine	2.21		
Tyrosine	1.59		
Valine	2.77		
Tryptophan	0.56		

[1]Analysis was determined by All-tech®, Nicholasville, KY 40356.

[2]NFE = Nitrogen free extract.

[3]TSAA = Methionine + Cystine.

Table 2.3. Composition of the dietary treatments (as-fed basis). Experiment 2.

Ingredient, %	Starter (0 to 14 d)				Grower (15 to 32 d)
	Control	0.25% *Torula yeast* RNA[1]	2% NT[2] product[3]	6% NT[2] product[3]	All treatments
Corn	60.31	59.73	60.58	61.53	64.16
Soybean meal (49%)	33.51	33.68	31.46	27.13	30.72
Poultry Fat	2.36	2.52	2.14	1.65	2.21
Torula yeast RNA[1] or NT[2] product[3]	-	0.25	2.00	6.00	-
Limestone	1.13	1.13	1.13	1.13	0.61
Dicalcium phosphate	1.73	1.73	1.73	1.60	1.46
Salt	0.35	0.35	0.35	0.35	0.37
Vitamin Mix[4]	0.25	0.25	0.25	0.25	0.25
Mineral mix[5]	0.08	0.08	0.08	0.08	0.08
DL-Metionine	0.20	0.20	0.20	0.20	0.14
L-Lys-HCL (79%)	0.08	0.08	0.08	0.08	-
Contents by calculation					
ME_N, kcal/kg	3.082	3,080	3,080	3,080	3,108
Crude protein, %	21.0	21.0	21.0	21.0	20.0
Calcium, %	0.91	0.91	0.92	0.92	0.85
Avail. phosphorus, %	0.45	0.45	0.46	0.46	0.40

[1]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[2]NT = Nucleotides.

[3]Nupro®, All-tech®, Nicholasville, KY, 40356.

[4]Vitamin mix provided the following (per kg of diet) : Thiamin mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12mg; vitamin B_{12}(cobalamin), 12.0µg; pyridoxine HCL, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5µg; trans-retinyl acetate, 1,892µg; all-rac α tocopheryl acetate, 11mg; ethozyquin, 125mg.

[5]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; idodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.

Table 2.4. Calculated digestible amino acid and nucleotide content of the dietary treatments (as-fed basis). Experiment 2.

	Starter (0 to 14 d)				Grower (15 to 32 d)
Digestible amino acids, %	Control	0.25% *Torula yeast* RNA[1]	2% NT[2] product[3]	6% NT[2] product[3]	All treatments
Arginine (1.18)[4]	1.29	1.29	1.26	1.20	1.28 (0.96)[4]
Histidine (0.39)	0.53	0.53	0.52	0.51	0.48 (0.31)
Isoleucine (0.75)	0.96	0.97	0.92	0.81	0.77 (0.61)
Leucine (1.22)	1.73	1.73	1.71	1.67	1.63 (0.97)
Lysine (1.12)	1.10	1.10	1.10	1.10	0.97 (0.89)
TSAA[5] (0.81)	0.80	0.80	0.80	0.79	0.75 (0.67)
Phe+Tyr[6] (1.18)	1.70	1.70	1.68	1.63	1.38 (0.93)
Threonine (0.75)	0.74	0.74	0.74	0.74	0.68 (0.62)
Valine (0.86)	1.05	1.05	1.04	1.03	0.87 (0.71)
Calculated total Nucleotides[7]	0.370	0.522	0.422	0.524	-

[1]Ribonucleic acid from Torula yeast Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[2]NT = Nucleotides.

[3]Nupro®, All-tech®, Nicholasville, KY, 40356.

[4]Digestible amino acid recommendations for starter and grower broiler chickens (Baker, 1997).

[5]TSAA = Methionine +Cystine.

[6]Phe + Tyr = Phenylalanine + Tyrosine.

[7]Calculations based on the analysis from Chemoforma Ltd Rheinstrasse 28-32 Augst BL, Switzerland.

Table 2.5. Performance results of broilers fed diets with varying levels of *Torula yeast* RNA from 0 to 16 d of age. Experiment 1[1].

Parameter	Days of age	Treatments			SEM
		Control	0.25% *Torula yeast* RNA[2]	0.50% *Torula yeast* RNA[2]	
BW gain, g/chick	0 to 3	25	28	27	2.2
	4 to 8	89	85	89	2.3
	9 to 16	316	316	331	6.8
	0 to 16	430	430	447	9.3
Feed intake, g/chick	0 to 3	22	24	23	1.7
	4 to 8	103	104	105	3.2
	9 to 16	536	519	552	14.2
	0 to 16	660	647	680	14.2
Feed : gain, g/g	0 to 3	0.88	0.85	0.84	0.04
	4 to 8	1.16	1.22	1.18	0.03
	9 to 16	1.70	1.65	1.67	0.06
	0 to 16	1.54	1.51	1.52	0.04

[1]Means represent 6 replications per treatment, 10 chicks per replication.

[2]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

Table 2.6. Physical development of the small intestine of broilers fed diets with varying levels of *Torula yeast* RNA on 3, 8 and 16 d of age. Experiment 1[1].

Parameter		Days of age	Treatments			SEM
			Control	0.25% *Torula yeast RNA*[2]	0.50% *Torula yeast RNA*[2]	
Relative weight, g/kg of BW	Duodenum	3	16.6	17.5	17.4	1.62
		8	19.4	19.7	19.0	1.30
		16	14.3	14.6	14.2	0.58
	Jejunum	3	26.2	25.7	22.9	1.23
		8	29.1	28.7	29.3	1.76
		16	21.9	22.6	23.3	0.71
	Ileum	3	17.7	19.3	22.6	1.50
		8	23.8	24.9	23.8	2.04
		16	15.0	15.5	15.4	0.67
Relative length, cm/kg of BW	Duodenum	3	186	177	178	8.4
		8	102	101	101	5.3
		16	45	46	45	2.0
	Jejunum	3	397	370	349	16.4
		8	233	218	214	11.4
		16	97	100	99	3.4
	Ileum	3	357	333	354	23.4
		8	218	210	201	11.5
		16	94	102	97	4.1

[1]Means represent 12 birds per treatment and a total of 48 birds per each measured day.

[2]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

Table 2.7. Performance[1] results of broilers[2] fed diets with *Torula yeast* RNA or a commercial nucleotide product from 0 to 14 d of age. Experiment 2.

Parameter	Days of age	Treatments				SEM
		Control	0.25% *Torula yeast RNA*[3]	2% NT[4] product[5]	6% NT product	
BW gain, g/chick	0 to 14	323[c]	334[b]	358[a]	345[b]	4.4
	15 to 32	1,295	1,328	1,323	1,312	7.9
	0 to 32	1,617[b]	1,668[ab]	1,681[a]	1,656[ab]	17.4
Feed intake, g/chick	0 to 14	408	407	413	411	2.1
	15 to 32	2,075	2,123	2,119	2,075	10.4
	0 to 32	2,482	2,529	2,531	2,486	20.5
Feed : gain, g/g	0 to 14	1.26[b]	1.20[a]	1.15[a]	1.19[a]	0.02
	15 to 32	1.60	1.60	1.60	1.58	0.01
	0 to 32	1.54[b]	1.52[ab]	1.51[a]	1.50[a]	0.01

[a,b]Means within a row and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means represent 6 replications per treatment, 56 chicks per replication.

[2] The birds were fed the diets supplemented with *Torula yeast* RNA or a commercial nucleotide product until 14 d of age. All the birds were fed the same standard grower diet (with no supplementation of nucleotide from 15 to 32 d of age.

[3]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[4]NT = Nucleotides.

[5]Nupro®, All-tech®, Nicholasville, KY, 40356.

Table 2.8. Jejuna morphology[1] result of broilers fed diets with *Torula yeast* RNA or a commercial nucleotide product on 14 d of age. Experiment 2.

Morphology	Treatments				SEM
	Control	0.25% *Torula yeast* RNA[2]	2% NT[3] product[4]	6% NT[3] product[4]	
Villous height, µm	649[c]	747[a]	707[b]	660[c]	11.2
Crypt depth, µm	143	143	143	141	2.9
Villous height : Crypt depth ratios	4.8[b]	5.4[a]	5.2[a]	4.8[b]	0.14

[a,b]Means within a row and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means of 54 measurements per treatment (9 measurements for each jejuna sample × 6 birds per treatment, a total of 24 birds).

[2]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[3]NT = Nucleotides.

[4]Nupro®, All-tech®, Nicholasville, KY, 40356.

CHAPTER 3

EFFECT OF DIETARY NUCLEOTIDE SUPPLEMENTATION ON PERFORMANCE AND ON GASTROINTESTINAL TRACT DEVELOPMENT AND MORPHOLOGY OF BROILERS CHALLENGED WITH COCCIVAC-B® [1]

[1] Jung, B. and A. B. Batal. To be submitted to *Poultry Science*.

ABSTRACT

An experiment was conducted to determine the effects of dietary nucleotide supplementation on performance and on intestinal tract development and morphology when birds were exposed to a *Coccidiosis* challenge and placed in an increasing stocking density condition. Five hundred and twenty 1-d-old Cobb 500 male broiler chicks were placed in floor pens with previously used pine wood shavings (1 flock) at a stocking density 17.5 birds/m^2. Five replicate pens of twenty six chicks were randomly allotted to each of four dietary treatments. The experiment was a 2 × 2 factorial; two supplemental levels (0 and 0.25%) of *Torula yeast* RNA as a source of nucleotides and either a challenge with a high-dose Coccivac-B® vaccine at 4 d of age or no challenge. The birds were only fed the diet supplemented with nucleotides until 13 d of age after which time all the birds were fed a common grower diet until 31 d of age. Performance was measured on 9, 13, 23, and 31 d of age, and intestinal weight, morphology, and serum citrulline levels were measured on 13 d of age. From 10 to 13 d of age (6 to 9 d post-challenge), the birds challenged with Coccivac-B® vaccine had significantly poorer performance than the non-challenged birds. The addition of 0.25% *Torula yeast* RNA to the diet significantly increased body weight (BW) gain and feed efficiency (gain:feed) as compared to the control diet when the birds were challenged with the Coccivac-B® vaccine. Overall, from 0 to 31 d of age, the birds challenged with Coccivac-B® vaccine compensated in growth rate and had the same body weight gain as the non-challenged birds. The birds challenged with Coccivac-B® vaccine had significantly heavier jejunum and ileum relative weight (g/kg of BW) as compared to the non-challenged birds. The levels of citrulline in the serum were significantly decreased due to the *Torula yeast* RNA addition in bird diets both challenged and non-challenged. In conclusion, the addition of 0.25% *Torula yeast* RNA (as a nucleotide source) to the diet improved the

performance of birds challenged with Coccivac-B® vaccine and reared in the increased stocking density from 10 to 13 d of age (6 to 9d post-challenge).

(Key Words: Nucleotides, broilers, intestinal weight, Coccivac-B®)

INTRODUCTION

Nucleotides are the basic units of ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs). Nucleotides play a role in various physiological reactions that are important in maintaining life (Mateo, 2005). They are a source of cellular energy especially, adenosine 5'triphosphate (ATP) and are involved in lipid metabolism and protein synthesis (Cosgrove, 1998). Nucleotides also affect growth, development of gastrointestinal tract, and development of the immune system, stimulating production of IL-12, interferon-γ, and immunoglobulin G (IgG) (Carver and Walker. 1995; Nagafuchi *et al*., 1997; Jyonouchi *et al*., 2001). Nucleotides can be synthesized within cells from amino acids and glucose by a *de novo* pathway. They are also recycled through a salvage pathway where nucleotides are resynthesized from nucleosides that originate from nucleotide catabolism or dietary sources. Nucleotides are not considered an essential nutrient because they can be synthesized or salvaged at sufficient levels believed to meet the requirements of the body under normal conditions. However, recently researchers have noted that both mechanisms may not produce enough nucleotides in order to maintain normal functions under certain conditions, such as rapid growth, reproduction, malnutrition, or recovery from injuries in animals and human (Yu, 1998; Carver, 1999).

Studies have investigated the effects of dietary nucleotide supplementation on performance and development of gastrointestinal tract in birds reared in previously used litter (Jung and Batal, 2011) and in pigs infected with *Escherichia (E.) coli* (Maribo, 2003). Jung and Batal (2011) found that the supplementation of 0.25% *Torula yeast* RNA (containing 62% total nucleotide content) or 2% Nupro® (containing 7% total nucleotide content) to a corn-soybean meal based broiler diet significantly increased BW gain, feed efficiency, relative jejuna weight, and jejuna villous height of birds reared in previously used litter. Maribo (2003) reported that the

supplementation of a commercial yeast extract product (Nupro® ; containing 7% total nucleotide content) increased BW gain and improved feed conversion ratios as compared to the non-supplemented control diet when pigs were infected with *E. coli.* Cruickshank (2002) and Maribo (2003) suggested that stress conditions, such as environmental change or a disease challenge, such as *Coccidiosis* or *E. coli*, are required to observe any positive effects on animal performance from yeast products that contain high nucleotides.

Coccidiosis is a parasitic disease of the intestinal tract of animals, caused by intracellular protozoan parasites that belong to the genus *Eimeria*. This infection can cause diarrhea, bloody feces and reduced BW gain and feed efficiency in birds (Williams, 2002). *Eimeria acervulina* infections lead to several gastrointestinal morphological changes; mucosal soughing, truncated and thickened villous, increase in crypt depth, epithelial cell turnover, inflammation, and increased relative length and weight of intestine (Allen 1984 and 1987). High stocking density can lead to a reduction in bird performance due to reduced airflow between birds, or high environmental temperature, and increased numbers of *Coccidia* oocysts in the litter (Burkholder *et al*., 2008: Puron *et al*., 1995: Lillehoj and Lillehoj, 2000). Therefore, we hypothesized that a *Coccidiosis* challenge combined with an increased stocking density could be used to provide effective experimental stress conditions to reduce performance and alter intestinal morphology of birds during the first 31 d of life, when rapid development of the digestive tract is occurring.

The objective of the current study was to determine the effects of dietary nucleotide supplementation on performance, intestinal tract development and morphology, and serum levels of citrulline (an indicator of enterocyte mass) of broilers challenged with Coccivac-B® combined with an increased stocking density.

MATERIALS AND METHODS

Birds and treatments

All experimental protocols were reviewed and approved by the Animal Care and Use Committee at the University of Georgia. In a 2 × 2 factorial arrangement, five hundred and twenty 1-d-old Cobb 500 male broiler chicks were randomly assigned to two experimental diets (0 or 0.25% *Torula yeast* RNA) in the presence or absence of Coccivac-B® challenge at 4 d of age. Chicks were randomly assigned to 20 pens of 26 birds each. Treatments were applied such that these were 5 replicates of each treatment combination. Floor pens were equipped with hanging tube feeders and a Ziggity nipple drinker (Ziggity Systems Inc., Milford, IN) having 5 nipples. Floors were covered with previously used pine wood shavings (1 flock prior). Pens were 1.22 m × 1.22 m pen providing a density of 17.5 birds/m^2. Space per bird was considered to be an increased stocking density. The suggested stocking density was 14.3 birds/m^2 (Feddes *et al*., 2002). Chicks were maintained on a 24 h light, 1 h dark schdule for first 3 d and then a 23 h lighting schedule in a thermostatically controlled room. Corn-soybean meal mash experimental diets were formulated to be isocaloric and isonitrogenous at 3,000 kcal ME/kg and 20 % crude protein (CP). Birds were fed either the non-supplemented control or nucleotide supplemented diet from 0 to 13d of age, followed by a pelleted common grower diet (3,108 kcal ME/kg and 20 % CP) with no supplementation of nucleotides (Table 3.1) from 14 to 31 d of age. Chicks had *ad libitum* access to feed and water from 0 to 31 d of age. Calculated digestible lysine, total sulfur amino acid (TSAA) and threonine content in the two dietary treatments fed during the starter period (0 to 13 d of age) were approximately 5% lower than Baker's (1997) recommendations whereas the calculated digestible content of all the other amino acids exceed Baker's (1997) recommendations for the grower period (Table 3.2). The total calculated nucleotide level in the

experimental diets was 0.36 for the control diet and 0.51% for the 0.25% *Torula yeast* RNA supplemented diet for the starter period (from 0 to 13 d of age) (Table 3.2). All diet samples were submitted to a commercial laboratory, All-tech®, Nicholasville, KY, for ribonucleic acid (RNA) quantification analysis (Table 3.2). Briefly, diet samples were extracted with perchloric acid and ammonium-dihydrogen-phosphate at 95℃ for an hour. Then the nucleotides were separated by high performance liquid chromatography (HPLC) and measured with a UV spectrophotometry at 265nm as described previously by Lassalas *et al.* (1993). Body weight (BW) gain and feed intake were evaluated on 9, 13, 23, and 31d of age (5, 9, 19, and 27 d post-challenge, respectively) and feed efficiency (gain:feed) was calculated. At the occurrence of mortality, feed intake was adjusted based on bird days on feed.

Coccivac-B® challenge and inoculation dose

Coccivac-B® (Intervet/Schering-Plough Animal Health, USA), a vaccine used for chickens to prevent *Coccidiosis*, contains live oocysts from multiple coccidian species (*Eimeria acervulina, E. maxima, E. mivati*, and *E. tenella*). The manufacturer recommended dose for this vaccine (Intervet/Schering-Plough Animal Health, USA) is approximately 1,400 oocysts per chick. Half of the birds from the control and half of the birds from the *Torula yeast* RNA supplemented diet group were challenged with a high-dose Coccivac-B® vaccine of approximately 69,750 oocysts per chick which is fifty times higher than vaccination dose. The dose was administrated in 1 ml saline solution directly into the crop via oral gavage at 4 d of age recommended by Dr. Mathis (a personal communication). Remaining birds received an equal volume of saline solution orally gavaged as a sham control.

Analysis of serum citrulline level

Two broiler chicks were randomly selected from each replicate pen (10 birds per treatment, a total of 40 birds) for analysis of serum citrulline level. At 13 d of age, blood samples were collected via cardiac puncture with 6ml syringe attached to a 18.5G needle and transferred to collection tubes. All the samples were allowed to clot for 1 hour at room temperature prior to centrifugation at 1,000 G for 5 minutes for serum separation. Each serum sample was aliquoted in a 1.5ml micro-centrifuge tube and stored at -20℃ until further analysis. Serum citrulline level was determined by the Boyde and Rahmatullah method (1980). Briefly, 100µl of serum was diluted 20-fold with the addition of 1,900µl of distilled water and then added to a 25% tricarboxylic acid (TCA) solution to remove serum protein. After centrifugation for 5 minutes at 80 G, 0.5 ml of supernatant was harvested and mixed with 1.5 ml of chromogenic solution by vortexing. The sample was then boiled at 100℃ for 5 minutes and cooled prior to spectrophotometer reading at 530 nm absorbance. DL-citrulline (25 - 300 nmol) served as a standard.

Analysis of intestinal relative weight and morphology

After bleeding for analysis of serum citrulline, 2 broiler chicks from each replicate pen (10 birds per treatment, a total of 40 birds) were weighed individually and then euthanized by cervical dislocation. The abdominal cavity was opened by a mid-line incision and the small intestine was removed and divided into three segments: duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the bile ducts to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction). Any intestinal contents were

removed from the intestinal segments by flushing with a 0.9% saline solution and the weight of the empty small intestine was recorded and relative weight was calculated (Miles *et al*., 2006).

For analysis of intestinal morphology, 2 broiler chicks from each replicate pen (10 birds per treatment, a total of 40 birds) were euthanized and the jejunum was taken at 13 d of age. After emptying and flushing the jejunum, approximately 5 cm of the middle portion of the jejunum was excised with surgical blade and fixed in 10% formalin solution. Three cross sections of 10% formalin-fixed, paraffin-embedded jejuna segments were sectioned at 3μm and stained with hematoxylin and eosin stain (Uni *et al*., 1995). A total of three, intact, well-oriented villi were selected with three replicates from each jejuna cross section (nine measurements for each jejuna sample with seventy two measurements per treatment). Intestinal epithelial villous height was measured from the tip of the villous to the basement membrane of the villous, and crypt depth was measured from the villous bottom to the crypt. Villous height and crypt depth ratios were calculated. Images were taken by computer-aided light microscopy (10 × magnification of the objective lens and 1 × magnification of the adapter lens) and morphological indices were analyzed using Image-pro plus version 3.0 image software.

Statistical analysis

All data were subjected to ANOVA procedure for completely randomized design (Steel and Torrie, 1980) using the general linear model procedure (PROC GLM) of SAS® software (SAS Institute, 2005). Statistical significance of differences among treatments was assessed using the Duncan's new multiple range test. All data were also analyzed to determine significance of main effects (diets and Coccivac-B® challenge) and interactions (diets ×

Coccivac-B® challenge). A probability level of $P < 0.05$ was used to determine statistical significance of differences among the treatments.

RESULTS AND DISCUSSION

Body weight (BW) gain, feed intake, and feed conversion (feed:gain) ratios results are summarized in Table 3.3. A high-dose Coccivac-B® challenge (50 × higher than the manufacturer recommendations) significantly decreased BW gain, feed intake, and feed efficiency (gain:feed) from 10 to 13 d of age (6 to 9 d post-challenge). A number of studies have reported that broilers infected with *Coccidiosis* have decreased performance during the acute phase of the infection (5 to 7 d post-infection) (Witlock and Ruff, 1977; Chapman *et al*., 1982; Conway *et al.,* 1993). In the current experiment, supplementation of 0.25% *Torula yeast* RNA to a diet did not affect the BW and feed intake of birds that were in high density pens and non-challenged or challenged with a high-dose of Coccivac-B® vaccine at any period measured in the current experiment. The supplementation of 0.25% *Torula yeast* RNA to the diets also did not affect feed efficiency (gain:feed) at any period measured except from 10 to 13d of age (5 to 7d post-challenge). The supplementation of 0.25% *Torula yeast* RNA numerically ($P = 0.056$) improved gain:feed ratios regardless of Coccivac-B® challenge and significantly ($P < 0.05$) improved gain:feed ratios when birds were challenged with a high-dose of Coccivac-B® vaccine from 10 to 13 d of age. Positive effects on performance due to the addition of nucleotides to diet under stresses condition have been observed previously in birds (Jung and Batal, 2011) and pigs (Maribo, 2003). Supplementation of 0.25% *Torula yeast* RNA (containing 62% total nucleotide content) and 2 or 6% nucleotide product, Nupro® (containing 7% total nucleotide content), significantly increased feed conversion (feed:gain) ratios in birds under stress conditions induced

by previously used litter (2 flocks) from 0 to 14 d of age (Jung and Batal, 2011). Maribo (2003) also reported that pigs infected with *Escherichia (E.) coli* and fed diets supplemented with a yeast extract (Nupro®; containing 7% of free nucleotides) as a source of nucleotide had a 4.0% increase in BW gain and improved feed conversion ratios as compared to the pigs fed the control diet. Esteve-Garcia *et al.* (2007) reported that broilers fed diets supplemented with a 0.05% nucleotide product (Nucleoforce®; contained 26.4% total nucleotides content) had improved feed:gain of birds under no obvious stress conditions from 0 to 42 d of age. However in the experiment reported here in, diets supplemented with 0.25% *Torula yeast* RNA did not affect the BW gain and feed efficiency of birds if they were not challenged with Coccivac-B® vaccine. Similarly, other researchers did not find clear effects of nucleotide supplementation on growth performance of birds. Deng *et al.* (2005) noted that the supplementation of 0.5% and 1.0% *Torula yeast* RNA product (containing 62% total nucleotide content) in laying hens diet did not affect performance from 0 to 12 weeks of age under normal conditions. Pelicia *et al*., (2010) also did not find any differences on BW and feed intake due to the addition of up to 0.07% nucleotides-rich product (containing 22.5% total nucleotide content) to a corn-soybean meal basis diet in birds reared in new litter and strict health control from 0 to 42 d of age. Differences among studies could be associated with the content of available nucleotides in the different nucleotide products or yeast RNA products. As expected, the lack of stress conditions in non-challenged birds in the current experiment may resulted in no effect of the supplementation of the nucleotide product. However, when birds were challenged with the Coccivac-B® vaccine the addition of 0.25% *Torula yeast* RNA to the diet significantly increased feed efficiency (gain:feed) as compared to the control diet. Cruickshank (2002) and Maribo (2003) suggested that stress conditions, such as environmental changes or challenge and infection stress is required

to see more beneficial effects on performance due to the supplementation of nucleotides-rich products in broilers or pigs.

The intestinal tract development changes are shown in Table 3.4. At 13 d of age (9 d post-challenge) the relative jejuna and ileum weights (g/kg of BW) of birds challenged with a high-dose Coccivac-B® vaccine were significantly heavier as compared to the non-challenged birds. Allen (1984) reported that an infection with *E. acervulina* (2×10^6 sporulated oocysts / bird) increased fresh weights of jejunum and ileum at 7 d post-infection when oocyst shedding was at its maximum. The increased weights of ileum and jejunum may be related to changed morphology; increased villi height and width, and increased mucosal weight and thickness due to cellular infiltration induced by parasitic infection (Allen, 1987; Raines, 1989). The supplementation of 0.25% *Torula yeast* RNA to the diets increased relative duodenum and ileum weight of birds as compared to the non-supplemented control diet at 13 d of age in the current experiment when birds were challenged with the high-dose Coccivac-B®. Meanwhile, there was no difference in relative small intestinal weights between birds fed the control or the 0.25% *Torula yeast* RNA supplemented diets if they were not challenged with Coccivac-B® vaccine.

The serum citrulline measurements are presented in Table 3.4. Citrulline is a nonessential free amino acid and the metabolic product of glutamine, glutamate, proline, and arginine. It is specifically synthesized by enterocytes in the small intestine (Wu and Morris, 1998; Windmueller and Spaeth, 1981; Wu *et al*., 1994). Plasma or serum citrulline concentrations are considered a biomarker of enterocyte function (Crenn *et al*., 2009), and are used for quantitative enterocyte mass assessment in villous atrophy disease, short bowel disease and Crohn's disease in humans (Crenn *et al*., 2003; Papadia *et al*., 2007). Researchers have found that patients with those diseases had low plasma or serum citrulline concentration. On the

other hand, citrulline concentration was not influenced by inflammatory status in intestine (Papadia *et al.*, 2007). Similarly, in the current experiment, challenging the birds with a high-dose of the Coccivac-B® vaccine, which cause inflammation in intestine, did not affect the level of serum citrulline measured at 13 d of age. The birds fed the diet supplemented with 0.25% *Torula yeast* RNA had significantly lower citrulline concentration as compared to birds fed control diet in both presence and absence of a Coccivac-B® challenge at 13 d of age (9 d post-challenge). Thus, enterocytes in the small intestine may not have to activate to synthesize citrulline when the birds were fed the dietary nucleotides. Feeding nucleotides may actually decrease maintenance cost in birds.

Based on the histopathology examinations, birds challenged with a high-dose of Coccivac-B® vaccine had shorter duodenum villous but had significantly longer villous in the jejunum and ileum than the un-challenged birds at 13 d of age (9 d post-challenge) (Table 3.5). Similarly, Witlock and Ruff (1977) reported that the birds infected with *E. maxima* had longer villi in the jejunum as compared to the birds that were not infected at 7 d post-infection. Increased villous height is indicative of increase surface, which may compensate damaged intestinal epithelial cells to absorb nutrients in the gut (Yason *et al.*, 1987). In the current experiment, the birds fed a diet supplemented with 0.25% *Torula yeast* RNA had shorter villi in duodenum, jejunum, and ileum as compared to the birds fed a non-supplemented diet when birds were not challenged with Coccivac-B® vaccine. When birds fed 0.25% *Torula yeast* RNA, challenged with a high-dose Coccivac-B® vaccine had significantly increased the villous height in the jejunum. Similarly, birds fed diets supplemented with 0.25% *Torula yeast* RNA (containing 62% total nucleotide content) and 2% Nupro® (containing 7% total nucleotide content) had significantly higher villous height in the jejunum as compared to birds fed control

diet under the stress conditions of previously used litter (Jung and Batal, 2011). In a swine study, the addition of a commercial nucleotide product (Nucleoforce Piglets® ; containing 25% free nucleotide content) at 1,000 and 2,000 ppm to early-weaned piglet diets increased villous height in the ileum as compared to piglets fed the control diet without nucleotide supplementation (Martinez-Puig *et al*., 2007). In the current experiment at 13 d of age (9 d post-challenge) birds challenged with the high-dose Coccivac-B® vaccine had significantly deeper crypt depth in the duodenum, jejunum, and ileum as compared to birds that were not challenged. The supplementation of 0.25% *Torula yeast* RNA significantly decreased crypt depth in the duodenum and ileum as compared to the control diet when the birds were challenged with the high-dose Coccivac-B® . However, if the birds were not challenged with Coccivac-B® vaccine the 0.25% *Torula yeast* RNA supplemented diet decreased only the crypt depths in duodenum as compared to the control diet. Cells in the crypt continually divide and provide the source of the epithelial cells on the villi. Deeper crypts indicate fast cell turnover, which is often observed in response to inflammation or infection to renew the damaged tissue (Yason *et al*., 1987). The supplementation of *Torula yeast* RNA as a source of nucleotides in the diet reversed the negative effect of Coccivac-B® challenge on crypt depth in duodenum. Crypt depth in jejunum was not affected by the supplementation of 0.25% *Torula yeast* RNA whether the birds were challenged with Coccivac-B® or not. Results from a study in which birds were under environmental stress, raised on previously used litter showed no difference in jejuna crypt depth between birds fed control and various nucleotide supplemented (0.25% *Torula yeast* RNA and 2 and 6% Nupro®) diets (Jung and Batal, 2011). Thus, crypt depth in jejunum may be less sensitive than that in duodenum or ileum to the addition of dietary nucleotide supplementation in broiler diets. The villous height and crypt depth ratio in duodenum and jejunum was low due to the high-dose

Coccivac-B® challenge. Regardless of Coccivac-B® challenge, the supplementation of the 0.25% *Torula yeast* RNA significantly increased villous height to crypt depth ratio in duodenum. In ileum, the high-dose Coccivac-B® challenge did not affect the ratio but, the 0.25% *Torula ye*ast RNA supplementation significantly decreased the ratio as compared to the control diet. This response resulted in Coccivac® -B challenge × *Torula yeast* RNA supplementation interaction for the villous height to crypt depth ratio in ileum ($P < 0.01$).

In conclusion, nucleotides are not considered an essential nutrient in poultry diets. However, based on the results from the current study, *Torula yeast* RNA as a source of nucleotides may be important to maintain maximum performance of birds that were challenged with the high-dose Coccivac-B® vaccine and reared in the increased stocking density conditions. In addition, 50 × dosage of Coccivac-B® challenge to birds at 4 d of age reduced performance, and cause physical and morphological changes in the gastro-intestinal track (GIT) without inducing significant clinical signs of *Coccidiosis* including mortality in broilers.

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Table 3.1. Composition of the dietary treatments (as-fed basis).

	Starter period[1] (0 to 13d)		Grower period (14 to 32d)
Ingredients, %	Control	0.25% *Torula yeast* RNA[1]	All treatments
Corn	65.00	64.49	64.16
Soybean meal (49%)	30.75	30.83	30.72
Poultry fat	0.40	0.58	2.21
Torula yeast RNA[1]	-	0.25	-
Limestone	1.13	1.13	0.61
Dicalcium phosphate	1.73	1.73	1.46
Salt	0.35	0.35	0.37
Vitamin Mix[2]	0.25	0.25	0.25
Mineral mix[3]	0.08	0.08	0.08
DL-Met	0.19	0.19	0.14
L-Lys-HCL (79%)	0.12	0.12	-
Total	100.0	100.0	100.0
Content by calculation			
TME, kcal/kg	3,000	3,000	3,108
Crude protein, %	20.0	20.0	20.0
Calcium, %	0.90	0.90	0.85
Avail. phosphorus, %	0.45	0.45	0.40

[1]Ribonucleic acid from Torula yeast Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[2]Vitamin mix provided the following (per kg of diet) : Thiamin mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12mg; vitamin B_{12}(cobalamin), 12.0µg; pyridoxine HCL, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5µg; trans-retinyl acetate, 1,892µg; all-rac α tocopheryl acetate, 11mg; ethozyquin, 125mg.

[3]Trace mineral mixes provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; iodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.

Table 3.2. Calculated digestible amino acid and nucleotide content of the dietary treatments (as-fed basis).

	Starter (0 to 13 d)		Grower (14 to 32 d)
Digestible amino acids, %	Control	0.25% *Torula yeast* RNA[1]	All treatments
Arginine (1.18)[2]	1.22	1.22	1.28 (0.96)[2]
Histidine (0.39)	0.50	0.50	0.48 (0.31)
Isoleucine (0.75)	0.91	0.91	0.77 (0.61)
Leucine (1.22)	1.68	1.68	1.63 (0.97)
Lysine (1.12)	1.07	1.07	0.97 (0.89)
TSAA (0.81)	0.77	0.77	0.75 (0.67)
Phe+Tyr (1.18)	1.62	1.62	1.38 (0.93)
Threonine (0.75)	0.70	0.70	0.68 (0.62)
Valine (0.86)	1.00	1.00	0.87 (0.71)
Calculated total nucleotide content[3]	0.36	0.51	-
Analyzed total RNA content[4]	1.13	1.48	

[1]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[2]Digestible amino acid recommendations for starter and grower broiler chickens (Baker, 1997).

[3]Total nucleotide content in each treatment diet was calculated based on the content of total nucleotide in each ingredient analyzed from Chemoforma Ltd. Rheinstrasse 28-32 Augst BL, Switzerland.

[4]Diet samples were sent to All-tech® , Nicholasville, KY, 40356 for RNA quantification analysis.

Table 3.3. Effects of feeding *Torula yeast* RNA as a nucleotide source on BW gain[1], feed intake[1], and feed efficiency (gain:feed) [1] of birds challenged with Coccivac-B® vaccine at 4 d of age from 0 to 31 d of age.

	Weight gain (g/chick)				Feed intake (g/chick)				gain:feed (g/kg)			
Treatments	0 to 9	10 to 13	14 to 31	0 to 31	0 to 9	10 to 13	14 to 31	0 to 31	0 to 9	10 to 13	14 to 31	0 to 31
No challenge, control	163	154[a]	1,196	1,513	208	221	1,908	2,337	784	696[ab]	627	647
No challenge, *Torula yeast* RNA	158	154[a]	1,203	1,515	209	217	1,900	2,327	757	709[a]	633	651
Challenge, control	166	128[b]	1,200	1,494	217	206	1,899	2,322	766	623[c]	633	644
Challenge, *Torula yeast* RNA	159	137[b]	1,189	1,485	213	206	1,883	2,302	749	664[b]	632	645
Pooled SEM	3.3	4.7	22.4	27.5	6.8	5.5	37.8	42.6	21.8	13.2	6.2	6.0
Challenges												
No challenge	161	154[a]	1,200	1,514	209	219[a]	1,904	2,332	770	703[a]	630	649
Challenge	162	133[b]	1,195	1,490	215	206[b]	1,891	2,312	757	643[b]	632	645
Pooled SEM	2.3	3.3	15.8	19.4	4.8	3.9	26.7	30.1	15.4	9.3	4.4	4.2
Diets												
Control	164	142	1,198	1,505	212	214	1,904	2,330	776	664	629	646
Torula yeast RNA	159	146	1,197	1,502	211	212	1,893	2,316	753	689	633	649
Pooled SEM	2.3	3.3	15.7	19.3	4.8	3.9	26.5	30.0	15.3	9.2	4.3	4.2
Source of variation				----------		P		----------				
Challenges	0.676	0.001	0.835	0.391	0.371	0.039	0.731	0.654	0.558	0.001	0.774	0.449
Diets	0.010	0.353	0.921	0.898	0.793	0.759	0.766	0.730	0.315	0.056	0.668	0.681
Challenges × Diets	0.774	0.372	0.688	0.835	0.689	0.706	0.914	0.912	0.826	0.298	0.557	0.835

[a-c] Means within a column and a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means represent 5 replications per treatment, 26 birds per replication, and 130 birds per treatment.

Table 3.4. Intestinal relative weight (g/kg of BW) [1] and serum citrulline levels[1] of birds fed diets supplemented with *Torula yeast* RNA and challenged with a high dose of Coccivac-B® vaccine at 4 d of age.

Treatments	13 d of age			
	Relative weight (g/kg of BW)			Serum citrulline, μM
	Duodenum	Jejunum	Ileum	
No challenge, control	21.7[b]	25.9[b]	18.6[c]	508[a]
No challenge, *Torula yeast* RNA	21.8[b]	26.2[b]	18.0[c]	268[b]
Challenge, control	24.9[a]	29.4[a]	24.6[a]	472[a]
Challenge, *Torula yeast* RNA	22.2[b]	30.4[a]	22.1[b]	278[b]
Pooled SEM	0.91	1.05	0.73	47.9
Challenges				
No challenge	21.7	26.1[b]	18.3[b]	388
Challenge	23.5	29.9[a]	23.3[a]	375
Pooled SEM	0.64	0.74	0.51	33.9
Diets				
Control	23.3	27.7	21.6[a]	490[a]
Torula yeast RNA	22.0	28.3	20.0[b]	273[b]
Pooled SEM	0.64	0.74	0.51	33.9
Source of variation	----------		P	----------
Challenges	0.053	0.001	0.001	0.784
Diets	0.163	0.538	0.044	0.001
Challenges × Diets	0.140	0.757	0.225	0.627

[a-c] Means within a column and a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means represent 10 birds per treatment and a total of 40 birds.

Table 3.5. Effects of feeding *Torula yeast* RNA as a nucleotide source on villous height[1], crypt depth[1], and villous height and crypt depth ratios[1] of broilers challenged with a high dose of Coccivac-B® vaccine at 4 d of age.

Treatments	13d of age								
	Villous height, μm			Crypt depth, μm			Villous height : crypt depth ratio, μm : μm		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
No challenge, control	1,053^{a}	674^{b}	388^{b}	207^{b}	182^{b}	127^{c}	5.4^{b}	4.0^{a}	3.3^{a}
No challenge, *Torula yeast* RNA	995^{b}	604^{c}	300^{c}	172^{c}	170^{b}	118^{c}	6.0^{a}	4.0^{a}	2.7^{b}
Challenge, control	965^{b}	688^{b}	474^{a}	242^{a}	212^{a}	171^{a}	4.2^{d}	3.4^{b}	3.0^{ab}
Challenge, *Torula yeast* RNA	962^{b}	764^{a}	396^{b}	214^{b}	207^{a}	144^{b}	4.8^{c}	3.9^{ab}	3.0^{ab}
Pooled SEM	17.5	16.1	9.7	5.6	5.0	4.3	0.18	0.17	0.12
Challenges									
No challenge	1,024^{a}	641^{b}	346^{b}	189^{b}	176^{b}	122^{b}	5.7^{a}	4.0^{a}	3.0
Challenge	963^{b}	724^{a}	434^{a}	226^{a}	210^{a}	157^{a}	4.5^{b}	3.6^{b}	3.0
Pooled SEM	12.4	11.4	6.9	4.0	3.6	3.0	0.13	0.12	0.08
Diets									
Control	1,012	684	434^{a}	223^{a}	197	150^{a}	4.8^{b}	3.7	3.1^{a}
Torula yeast RNA	978	685	355^{b}	194^{b}	189	133^{b}	5.4^{a}	3.9	2.8^{b}
Pooled SEM	12.4	11.4	6.8	4.0	3.6	3.0	0.13	0.12	0.08
Source of variation			----------		P	----------			
Challenges	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.051	0.978
Diets	0.088	0.836	0.001	0.001	0.115	0.001	0.001	0.261	0.005
Challenges × Diets	0.119	0.001	0.604	0.518	0.486	0.028	0.967	0.168	0.010

[a,b] Means within a column and a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means of 90 measurements per treatment (9 measurements for each jejuna sample × 10 birds per treatment, a total of 40 birds).

CHAPTER 4

EFFECT OF DIETARY NUCLEOTIDE SUPPLEMENTATION AND COCCIVAC-B® CHALLENGE ON BROILER PERFORMANCE AND NUTRIENT UTILIZATION[1]

[1] Jung, B. and A. B. Batal. To be submitted to *Poultry Science*.

ABSTRACT

An experiment was conducted to determine the effects of two dietary products that are high in nucleotides (*Torula yeast* RNA and Nupro®) and a nucleotide precursor (glutamine) on broiler performance and nutrient utilization (nitrogen corrected apparent metabolizable energy; AME_N) in broilers challenged with Coccivac-B® vaccine from 4 to 18 d of age. Five hundred and seventy six 4-d-old Cobb 500 male broiler chicks were placed in starter batteries in 9 replicate pens containing 8 chicks each and were randomly assigned to the 8 experimental treatments. The experiment was a 4 × 2 factorial arrangement (4 dietary treatments × 2 challenges). The four dietary treatments were: (1) a corn-soybean meal based control diet, (2) the control diet supplemented with 0.25% *Torula yeast* RNA, (3) the control diet supplemented with 2% Nupro®, and (4) the control diet supplemented with 1% glutamine. A high-dose (50× higher than recommended vaccination dose) of Coccivac-B® vaccine was superimposed on the dietary treatments at 4 d of age. Performance was measured at 8, 12 and 18 d of age (4, 8 and 14 d post-challenge) and excreta were collected for AME_N determination of the experimental diets at 12 and 18 d of age (8 and 14 d post-challenge, respectively). Overall (4 to 18 d of age) when birds were not challenged, the supplementation of 0.25% *Torula yeast* RNA and 2% Nupro® diets significantly increased body weight (BW) gain as compared to the control diet. When the birds were challenged with the high-dose of Coccivac-B® vaccine, the supplementation of 2% Nupro® diet significantly increased BW gain as compared to the control diet. The high-dose Coccivac-B® challenge decreased BW gain and feed intake of birds at all periods measured. From 4 to 8 d of age, non-challenged birds fed the diet supplemented with 0.25% *Torula yeast* RNA and 2% Nupro® had significantly better feed efficiency (gain:feed) than the non-challenged birds fed the control diet. While, when the birds were challenged with the high-dose Coccivac-B® vaccine, the

diet supplemented with 0.25% *Torula yeast* RNA significantly improved gain:feed ratios as compared to the control diet. The birds challenged with Coccivac-B® vaccine had poorer feed efficiency than the non-challenged birds from 4 to 18 d of age. The 0.25% *Torula yeast* RNA or 2% Nupro® supplementation increased the determined AME_N value of the experimental diets measured at 12 d of age as compared to the value from the control diet or 1% glutamine supplemented diet when the birds were challenged with the high-dose Coccivac-B® vaccine. The determined AME_N values of the experimental diets fed to the birds challenged with the high-dose Coccivac-B® were lower than that determined from the birds not challenged at 12 d of age. An interaction between diets × a high-dose Coccivac-B® challenge was observed at 18 d of age. A moderate positive correlation ($r^2 = 0.45$) was observed between the performance and AME_N value of experimental diets at 12 d of age (8 d post-challenge). In conclusion, the dietary nucleotide (0.25% *Torula yeast* RNA and 2% Nupro®) supplementation was effective at alleviating the negative effects of *Coccidiosis* infection on broiler growth performance and AME_N value of the experimental diets. However, the supplementation of a nucleotide precursor, glutamine, did not appear to replace nucleotides in broilers.

(**Key words**: Nucleotides, performance, AME_N)

INTRODUCTION

Nucleotides are important molecules in the body and are involved in various physiological reactions that are essential in maintaining life and reproduction (Mateo, 2005). Nucleotides are involved in lipid metabolism, protein synthesis, and energy metabolism. They also have an effect on growth as well as development of the gastrointestinal tract and immune system (Voet and Voet, 2004; Uauy, 1994). Adenosine 5'triphosphate (ATP) is the most common nucleotide as it serves as an energy-rich precursor in various cells (Carver and Walker, 1995). The majority of nucleotides are synthesized by a *de novo* pathway from amino acid precursors such as glutamine, formate, glycine, and aspartic acid. Nucleotides can also be recycled by a salvage pathway in which nucleotides are synthesized from intermediates generated during the breakdown of nucleotides (Carver and Walker, 1995). However, over the past several years, various researchers have concluded that the two aforementioned mechanisms may not provide enough nucleotides to maintain normal biological needs under certain conditions such as, rapid growth, reproduction, malnutrition, recovery from injuries, environmental change or disease challenge (Yu, 1998; Carver, 1999). Because of those reasons, they have been referred to as conditional essential nutrients in animals (Yu, 1998).

A small number of studies have indicated that the addition of nucleotides or nucleotide-rich yeast extract products to the diets did not affect performance in birds and swine raised under normal stress conditions. Deng *et al*. (2005) reported that the supplementation of 0.5 or 0.1% *Torula yeast* RNA (containing 62% total nucleotide content) from 0 to 4 weeks of age did not affect BW gain and feed:gain ratios of over the entire study (0 to 12 weeks of age) in Leghorn chickens reared under standard conditions. Andres-Elias *et al*. (2007) and Martinez-Puig *et al*. (2007) also reported that there were no significant effects on the performance due to the addition

of 0.10, 0.15, or 0.20% nucleotide-rich yeast extract containing 25% free nucleotides to the diets in weaned pigs reared under standard conditions. While, it has been reported that some stress conditions such as disease challenge in birds or pigs cause a positive response when birds are fed yeast products containing high nucleotide content (Cruickshank, 2002; Maribo, 2003). *Coccidiosis* is a parasitic disease in the intestinal tract of animals and caused by *Eimeria species* (Lillehoj and Lillehoj, 2000). *Coccidial* infection has been reported to reduce performance due to the disruption of intestinal function (Lillehoj and Lillehoj, 2000). This infection has been shown to decrease the rate of glucose absorption in the small intestine and reduce nitrogen corrected metabolizable energy (ME_N) and amino acid (AA) digestibility of the diets fed in chicks (Persia *et al*., 2006; Ruff and Wilkins, 1980; Preston-Mafham and Sykes, 1970). Thus, *Coccidial* infection was selected as a challenge in the current study to see if nucleotides could improve performance in stressed birds.

To date no research has been conducted to test the effects of a *Coccidiosis* challenge on the nutrient utilization of a corn-soybean meal based diet supplemented with dietary nucleotides or a nucleotide precursor in birds. Therefore, the objective of this study was to determine the effects of two nucleotide products, *Torula yeast* RNA and Nupro®, a nucleotide precursor, glutamine on broiler performance and nutrient utilization (nitrogen corrected apparent metabolizable energy; AME_N) in broilers challenged with Coccivac-B® vaccine from 4 to 18 d of age.

MATERIALS AND METHODS

Birds and treatments

All experimental protocols were approved by the Animal Care and Use Committee at the University of Georgia. Five hundred and seventy-six, one-d-old Cobb 500 male broiler chicks were obtained from a commercial hatchery. At 0 d of age the chicks were vaccinated for Marek's disease, Newcastle disease, and Infectious bronchitis. Chicks were housed in an environmentally controlled building and randomly distributed into thermostatically controlled Petersime starter batteries with trough type feeders and waterers. All birds were fed a commercial broiler starter diet (3,200 kcal ME/kg and 23 % crude protein) until starting the experiment (0 to 3 d of age). At 3 d of age, the birds were fasted overnight and chicks were weighed and assigned to treatment groups. All birds had *ad libitum* access to feed and water with a 24 h lighting schedule from 4 to 18 d of age. The eight experimental diets were assigned to nine replicate pens containing eight chicks each. The experiment was a 4 × 2 factorial arrangement (4 dietary treatments × 2 challenges). The four dietary treatments were: (1) a corn-soybean meal diet (control diet), (2) the control diet supplemented with 0.25% *Torula yeast* RNA, (3) the control diet supplemented with 2% Nupro®, and (4) the control diet supplemented with 1% glutamine. At 4 d of age, birds in half of pens from the each dietary treatment were inoculated with a high-dose (50× higher than recommended vaccination dose) of Coccivac-B® vaccine and the remaining birds were inoculated with an equal volume of saline. Four corn-soybean meal based experimental diets were formulated to be isocaloric and isonitrogenous at 3,000 kcal ME/kg and 20 % crude protein, respectively (Table 4.1.) Calculated digestible lysine, total sulfur amino acid (TSAA) and threonine content in all the treatment diets from 4 to 18 d of age were approximately 5% lower than Baker's (1997) recommendations, whereas all the other calculated digestible amino

acids met or exceeded Baker's (1997) recommendations (Table 4.2). Celite™[2] was added to all the experimental diets at 2% to serve as a source of insoluble ash (Table 4.1.). Calculated total nucleotide levels in the four treatment diets were 0.35, 0.51, 0.40, and 0.35% respectively. All diet samples were sent to All-tech®, Nicholasville, KY for ribonucleic acid (RNA) quantification analysis (Table 4.2). Briefly, cold 0.2N perchloric acid was added to individual feed samples and homogenated to remove acid-soluble small molecules followed by addition of 0.3N Potassium hydroxide (KOH) for one hour at 37℃ to solubilize the RNA in the sample with minimal release of peptides. Samples were then centrifuged at 3,000g for 20 minutes at 4℃. The supernatant that contains RNA was removed for high performance liquid chromatography (HPLC) analysis to determine the total RNA concentration (Munro and Fleck, 1969). Body weight (BW) gain and feed intake were measured on 8, 12, and 18 d of age (4, 8 and 14 d post-challenge) and feed efficiency (gain:feed) was calculated. At the occurrence of mortality, feed intake was adjusted based on bird days on feed.

Sample collection and AME_N value determination

Clean excreta trays were placed under each battery cage and excreta were collected for 48 h at 12 and 18 d of age (8 and 14 d post-challenge, respectively). The four experimental diets and all the excreta were dried at 55℃ for 48 h. The dried samples were then ground through a Thomas-Wiley mill[3] equipped with a 1-mm screen to ensure a homogeneous mixture. Samples were sent to a commercial laboratory[4] for analysis of gross energy (GE) and nitrogen content. Briefly, gross energy content of feed and excreta were determined with 2 grams of samples using

[2] Celite™, A diatomite product, Food Chemicals Codex Grade. Celite Corp., Lompar., CA 93436.

[3]Thomas-Wiley mill, Arthur H. Thomas Company, Philadelphia, PA.

[4]Minnesota Valley Testing Laboratories, New Ulm, MN.

an isoperbol oxygen bomb calorimeter[5] and analysis. Nitrogen content of feed and excreta was determined with a Combustion N analyzer[6] (AOAC, 1996). All analysies were tested twice. AME_N values of the each sample were calculated by the method described by Sibbald (1976). The AME_N was calculated using the following equation;

AME_N (kilocalories per kilogram of diet) = $GE_{diet} - [GE_{excreta} \times (Marker_{diet}/Marker_{excreta})] - [8.22 \times N_{diet} - [N_{excreta} \times (Marker_{diet}/Marker_{excreta})]$, where GE = gross energy [kcal/kg of sample (diet or excreta)], Marker = concentration of acid insoluble ash (Celite™) in diet or excreta, and 8.22 = nitrogen correction factor reported from previous research (Hill and Anderson, 1958).

Statistical analysis

All data were subjected to ANOVA procedure for randomized complete block design (Steel and Torrie, 1980) using the general lineal model procedure (PROC GLM) of SAS® software (SAS Institute, 2005). Statistical significance of differences among treatments was assessed using the Duncan's new multiple range test. All data were also analyzed by ANOVA to determine significance of main effects (diets and Coccivac-B® challenge) and interactions (diets × Coccivac®-B challenge). Correlation between performance and AME_N measured at 12 d of age was analyzed using the correlation procedure (PROC CORR) of SAS®. A probability level of $P < 0.05$ was used to determine statistical significance of differences among the treatments.

[5]Isoperbol oxygen bomb calorimeter, model 1281, Parr Instruments, Moline, IL.

[6]Combustion N analyzer, Truspec N Determinator, Leco Corp., St. Joseph, MI.

RESULTS AND DISCUSSION

Body weight (BW) gain, feed intake, and feed efficiency (gain:feed) results are summarized in Table 4.3. From 4 to 8 d of age or overall (from 4 to 18 d of age) when birds were not challenged with Coccivac-B® vaccine, 0.25% *Torula yeast* RNA, 2% Nupro®, and 1% glutamine supplementation diet significantly increased BW gain as compared to the control diet. While, when the birds challenged with the high-dose Coccivac-B® vaccine the 0.25% *Torula yeast* RNA and 2% Nupro® supplementation diet significantly increased BW gain as compared to the control diet from 4 to 8 d of age. Overall period (from 4 to 8 d of age) the only 2% Nupro® supplementation diet significantly increased BW gain. Regardless of challenge with the high-dose Coccivac-B® vaccine from 4 to 8 d of age or overall (from 4 to 18 d of age) the supplementation of the nucleotide products (0.25% *Torula yeast* RNA and 2% Nupro®) and a nucleotide precursor (1% glutamine) significantly increased BW gain as compared to the control diet. There was no difference in feed intake among all treatments at any periods measured when the birds were not challenged with Coccivac-B® vaccine. While, when the birds were challenged with the high-dose Coccivac-B® vaccine, the supplementation of 0.25% *Torula yeast* RNA or 2% Nupro® to the diets significantly increased feed intake as compared to the control diet from 4 to 8 d of age. Overall, from 4 to 18 d of age when high-dose Coccivac-B® challenged birds were fed a diet supplemented with either 2% Nupro® or 1% glutamine, feed intake significantly increased as compared to the birds fed the control diet. Regardless of challenge with a high-dose Coccivac-B® from 4 to 8 d or overall, from 4 to 18 d of age, the birds fed the diets supplemented with nucleotide products or the nucleotide precursor had higher feed intake than the birds fed the control diet. Feed intake was increased in birds fed 0.25% *Torula yeast* RNA supplemented diets from 9 to 12 d and 12 to 18 d of age as compared to the birds fed the control diet regardless of

Coccivac-B® challenge. The supplementation of 0.25% *Torula yeast* RNA and 2% Nupro® to the diet significantly improved feed efficiency (gain:feed) as compared to the control diet from 4 to 8 d of age when the birds were not challenged with a high-dose Coccivac-B® vaccine. While, if the birds challenged with the high-dose Coccivac-B® vaccine the only 0.25% *Torula yeast* RNA supplementation diet significantly improved feed efficiency as compared to the control diet. Regardless of challenge with the high-dose Coccivac-B® vaccine birds fed diets supplemented with 0.25% *Torula yeast* RNA and 2% Nupro® had improved feed efficiency (gain:feed) as compared to birds fed the control diet from 4 to 8 d of age. From 9 to 12 d of age birds fed diets supplemented with 0.25% *Torula yeast* RNA had improved feed efficiency (gain:feed) as compared to birds fed the control diet and 1% glutamine supplemented diet. From 12 to 18 d of age or overall, 4 to 18 d of age, there was no significant difference due to the addition of 0.25% *Torula yeast* RNA, 2% Nupro®, or 1% glutamine on the feed efficiency. Thus, the supplementation of two nucleotide products and the nucleotide precursor in diets had beneficial effects on performance of non-challenged or challenged birds with the high-dose Coccivac-B® vaccine. Nucleotides and a precursor of nucleotides, glutamine, are not considered as an essential nutrient for animals, because they can be synthesized by a *de novo* pathway and formed through a salvage pathway in the body. However, two aforementioned mechanisms cannot provide enough nucleotides to maintain optimal biological function in the body during periods of rapid growth or physical and physiological stress conditions such as, environmental change or disease challenge (Yu, 1998; Carver, 1999). Thus, the supplementation of nucleotides to the diets could optimize the function by sparing the cost of the *de novo* synthesis or salvage pathway during rapid growth, development of intestinal tract period and disease challenge. Some studies have indicated that providing supplemental nucleotides and glutamine in animal diets have positive

effects on performance during starter period, when rapid development of digestive tract is occurring. For example, birds fed diets supplemented with 0.05% commercial nucleotide product(Nuceloforce® ; containing 26.4% of balanced free nucleotides) were heavier and had improved feed conversion ratios as compared to birds fed the control diet with no supplementation of the nucleotide product from 0 to 21 d of age (Esteve-garci *et al*. 2007). Rutz *et al*., (2006) also demonstrated that the birds fed diet supplemented with 2% yeast extract (Nupro® ; containing 7% total nucleotide content) gained significantly more body weight during the starter period (0 to 14 d of age). In addition, this study was indicated that the supplementation of a yeast extract product to broiler diets increased feed intake, yet the differences were not significant. The authors mentioned that increased palatability of the diet was the reason for the increased feed intake. The supplementation of 1% glutamine, a nucleotides precursor, to broiler diet significantly increased BW gain from 0 to 14 d of age as compared to birds fed control diet (Bartell and Batal, 2007). When animals were raised in certain conditions, such as environmental or infectious disease challenges, the supplementation of nucleotides had also beneficial effects on performance. A study has been demonstrated that the supplementation of 0.25% *Torula yeast* RNA (containing 62% total nucleotide content) or 2% Nupro® (contained 7% total nucleotide content) to the diet significantly increased BW gain as compared to the control diet fed birds reared in certain conditions, such as dirty litter from 0 to 14 d of age (Jung and Batal, 2011). Stress conditions, such as environmental changes or challenge and infection stress are required to see more positive effects on performance due to the addition of yeast products (containing high nucleotide content) in broilers (Cruickshank, 2002) or pigs (Maribo, 2003). The challenging birds with a high-dose Coccivac-B® vaccine at 4 d of age had significantly lower BW gain and feed intake as compared to the un-challenging birds at all periods measured in the current

experiment. The birds challenged with a high-dose Coccivac-B® also had significantly worse feed efficiency (gain:feed) than the birds that were not challenged from 9 to 12 d or overall, 4 to 18 d of age. A number of studies demonstrated that *Coccidial* infections led to reduction in performance in birds due to the disruption of intestinal function (Lillehoj and Lillehoj, 2000; Conway *et al*., 1993. Persia *et al.*, 2006). In the current study the supplementation of 0.25% *Torula yeast* RNA or 2% Nupro® as a dietary nucleotide alleviated the negative effects of Coccivac-B® challenge on growth performance of broilers. Dietary nucleotides have beneficial effects on recovery of intestinal injuries from chronic diarrhea and infection disease (Carver and Walker, 1995). Maribo (2003) found improved performance by the diet supplemented with a yeast extract (Nupro®; containing 7% total nucleotide content) as a source of nucleotides in swine infected with *Escherichia (E.) coli*. The author demonstrated that the yeast extract supplementation to the swine diet at 4.0% resulted in improved BW gain, reduced incidence of diarrhea, and improved feed conversion ratios. No study has been conducted to test the effect of dietary nucleotides on performance of birds challenged with *Coccidiosis*. The mechanism of alleviating the negative effects of *Coccidiosis* challenge due to the supplementation of dietary nucleotides was not clear but, the intestinal injuries from Coccivac-B® vaccine in the birds might be partially recovered due to the nucleotide supplementation.

The nitrogen corrected apparent metabolizable energy (AME_N) of the experimental diets is summarized in Table 4.4. When birds are not challenged, no difference in the determined AME_N values of diets supplemented control, 0.25% *Torula yeast* RNA, and 2% Nupro® was observed whereas the values of 1% glutamine supplemented diet was lower than the other treatments at 12 d of age (8 d post-challenge). When the birds were challenged with the high-dose Coccivac-B®, the 0.25% *Torula yeast* RNA and 2% Nupro® supplemented diet significantly

increased the determined AME_N values of the diets as compared to the values from the control or 1% glutamine supplemented diet at 12 and 18 d of age (8 and 14 d post-challenge, respectively). The determined AME_N value of the diet that contain 0.25% *Torula yeast* RNA was significantly higher while the value of the diet containing 1% glutamine was lower than that determined for the control diet at 12 d of age (8 d post-challenge) regardless of the Coccivac-B® challenge. When birds were challenged with the high-dose Coccivac-B® vaccine or regardless of the challenge, the determined AME_N value of the diet supplemented with 0.25% *Torula yeast* RNA did not differ from the value of the control diet at 18 d of age (14 d post-challenge). Whereas, the AME_N values of the diet supplemented with 2% Nupro® or 1% glutamine were significantly lower than the values of the control diet or 0.25% *Torula yeast* RNA supplemented diet at 18 d of age (14 d post-challenge). The determined AME_N value of experimental diets fed to the birds challenged with a high-dose Coccivac-B® vaccine were lower than the AME_N values determined for the diet fed to the unchallenged birds at 12 d of age (8 d post-challenge) but not at 18 d of age (14 d post-challenge). The AME_N response to *Coccidiosis* infection of the chicks fed the diets is similar to previous reports (Persia *et al*., 2006; Ruff and Wilkins, 1980). The nucleotide products (*Torula yeast* RNA and Nupro®) reduced the negative effects of a high-dose Coccivac-B® vaccine on the determined AME_N of experimental diets at 12 d of age (8 d post-challenge) in the current experiment. The method of amelioration of *Coccidiosis* infection by nucleotides is unclear, but it is apparent that dietary nucleotide supplementation increased AME_N in birds that are challenged with Coccivac-B® vaccine. Nucleotides especially, ATP are involved in energy metabolism and is an energy-rich precursor that transports chemical energy within cells for metabolisms (Voet and Voet. 2004). The ATP also stimulates glucose production which used by most cells as a source of energy and a metabolic intermediate in the animals body (Carver and

Walker, 1995). Buxton *et al*., (1986) found that ATP infusion into perfused rat significantly increased glucose output from the liver as compared to the rat that was not infused with ATP. Thus, dietary nucleotide supplementation may have a positive effect on the determined AME_N of diets in birds challenged with *Coccidiosis*. Interaction between diets and Coccivac-B® challenge in AME_N value of diets was observed at 18 d of age (14 d post-challenge) because the challenge did not affect the determined AME_N of experimental diets but the supplementation of 2% Nupro® and 1% glutamine reduced the value of the diets in chicks as compared to the control diet.

In summary, supplementing nucleotide products and a nucleotide precursor to diets positively affected performance of bird non-challenged or challenged with the high-dose Coccivac-B® vaccine. The high-dose Coccivac-B® challenge (50 × higher than recommended vaccination dose) reduced the determined AME_N of diets from birds fed a corn-soybean meal control diet at 8 d post-challenge but not at 14 d post-challenge. The 0.25% *Torula yeast* RNA and 2% Nupro® significantly increased the determined AME_N of diets from birds challenged with the high-dose Coccivac-B® vaccine. Therefore, the dietary nucleotide (0.25% *Torula yeast* RNA and 2% Nupro®) supplementation were effective at alleviating the negative effects of *Coccidiosis* infection on broiler performance and AME_N value of diets. It is apparent that increased AME_N value of diets with supplemental levels of *Torula yeast* RNA and Nupro® resulted in increased growth performance. There was a moderate positive correlation ($r^2 = 0.45$; $P < 0.001$) between the performance and AME_N of experimental diets measured at 12 d of age (8 d post-challenge).

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Table 4.1. Composition of the dietary treatments (as-fed basis).

Ingredient, %	Pre-experimental period (0 to 3d)	Experimental period[1] (4 to 18 d)			
	All treatments	Control (T1 and T5)	*Torula yeast* RNA (T2 and T6)	Nupro® (T3 and T7)	Glutamine (T4 and T8)
Corn	55.5	61.1	60.4	61.4	59.0
Soybean meal (48%)	37.1	31.3	31.5	29.3	31.7
Poultry Fat	3.04	1.75	2.00	1.54	2.45
Celite™[2]	-	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.73	1.73	1.73	1.70	1.73
Limestone	1.23	1.13	1.13	1.12	1.13
Salt	0.29	0.35	0.35	0.35	0.35
Vitamin Mix[3]	0.25	0.25	0.25	0.25	0.25
Mineral mix[4]	0.74	0.08	0.08	0.08	0.08
DL-Met	0.20	0.19	0.19	0.19	0.19
L-Lys-HCL (79%)	-	0.12	0.12	0.12	0.11
Torula yeast RNA[5]	-	-	0.25	-	-
Nupro®[6]	-	-	-	2.00	-
Glutamine[7]	-	-	-	-	1.00
Contents by calculation					
TME, Kcal/kg	3,200	3,000	3,000	3,000	3,000
Crude protein, %	23.0	20.0	20.0	20.0	20.0
Calcium, %	1.00	0.90	0.90	0.90	0.90
Avail. phosphorus, %	0.45	0.45	0.45	0.45	0.45

[1]None-challenge group: T1, T2, T3, and T4; Challenged group by administration of Coccivac-B® at 4 d of age: T5, T6, T7, and T8.

[2]Celite™, a diatomite product, Food Chemicals Codex Grade. Celite Corp., Lompar., CA 93436.

[3]Vitamin mix provided the following (per kg of diet) : Thiamin·mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12mg; vitamin B_{12}(cobalamin), 12.0µg; pyridoxine·HCL, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5µg; trans-retinyl acetate, 1,892µg; all-rac α tocopheryl acetate, 11mg; ethozyquin, 125mg.

[4]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; idodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.

[5]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[6]Nupro®, All-tech®, Nicholasville, KY, 40356.

[7]L-Glutamine, Kyowa Hakko Kogyo Co., Ltd, 1-6-1, Ohtemachi, Chiyoda-ku, Tokyo, Japan.

Table 4.2. Calculated digestible amino acid and nucleotide content of the dietary treatments (as-fed basis).

Digestible amino acids	Experimental period[1] (4 to 18 d)			
	Control (T1 and T5)	*Torula yeast* RNA (T2 and T6)	Nupro® (T3 and T7)	Glutamine (T4 and T8)
Arginine (1.18)[2]	1.22	1.22	1.19	1.23
Histidine (0.39)	0.50	0.50	0.50	0.50
Isoleucine (0.75)	0.91	0.92	0.86	0.92
Leucine (1.22)	1.66	1.66	1.64	1.66
Lysine (1.12)	1.07	1.07	1.07	1.07
TSAA (0.81)	0.77	0.77	0.77	0.77
Phe+Tyr (1.18)	1.61	1.62	1.59	1.62
Threonine (0.75)	0.70	0.70	0.70	0.70
Valine (0.86)	0.99	1.00	0.99	1.00
Calculated total nucleotide content[3]	0.35	0.51	0.40	0.35
Analyzed total RNA content[4]	1.17	1.27	1.25	1.15

[1]None-challenge group: T1, T2, T3, and T4; Challenged group by administration of Coccivac-B® at 4 d of age: T5, T6, T7, and T8.

[2]Digestible amino acid recommendations for starter and grower broiler chickens (Baker, 1997).

[3]Ingredient samples were sent to Chemoforma Ltd. Rheinstrasse 28-32 Augst BL, Switzerland for nucleotides quantification.

[4]Ingredient samples were sent to All-tech® , Nicholasville, KY, 40356 for total RNA quantification.

Table 4.3. Effects of dietary nucleotide (*Torula yeast* RNA, Nupro®, and glutamine) supplementation on BW gain, feed intake, and feed efficiency (gain:feed)[1] of broilers non-challenged or challenged with a high-dose Coccivac®-B vaccine at 4 d of age.

	BW gain (g/chick)				Feed intake (g/chick)				Gain:feed (g/kg)			
Treatments	4 to 8	9 to 12	12 to 18	4 to 18	4 to 8	9 to 12	12 to 18	4 to 18	4 to 8	9 to 12	12 to 18	4 to 18
NC[2] + Control	107^{c}	156^{a}	340^{bc}	604^{b}	134^{abc}	216^{a}	481^{abc}	831^{a}	796^{d}	724^{a}	708	727^{a}
NC[2] + *Torula yeast* RNA	114^{a}	162^{a}	361^{a}	637^{a}	137^{ab}	221^{a}	503^{a}	862^{a}	830^{ab}	732^{a}	718	740^{a}
NC[2] + Nupro®	115^{a}	161^{a}	350^{ab}	625^{a}	138^{a}	219^{a}	490^{ab}	847^{a}	828^{abc}	736^{a}	715	739^{a}
NC[2] + glutamine	111^{ab}	156^{a}	351^{ab}	618^{ab}	138^{a}	221^{a}	487^{ab}	847^{a}	806^{cd}	705^{a}	727	732^{a}
C[3] + Control	105^{c}	68^{b}	317^{d}	490^{d}	131^{c}	146^{b}	441^{d}	717^{c}	804^{d}	465^{b}	719	683^{b}
C[3] + *Torula yeast* RNA	113^{a}	72^{b}	325^{cd}	510^{cd}	135^{ab}	149^{b}	455^{cd}	739^{bc}	839^{a}	481^{b}	715	690^{b}
C[3] + Nupro®	112^{ab}	76^{b}	333^{cd}	521^{c}	138^{a}	153^{b}	462^{bcd}	753^{b}	809^{bcd}	496^{b}	720	692^{b}
C[3] + glutamine	109^{bc}	71^{b}	326^{cd}	506^{cd}	134^{bc}	154^{b}	464^{bcd}	753^{b}	812^{bcd}	460^{b}	702	672^{b}
Pooled SEM	1.3	3.6	5.6	7.2	1.3	4.1	8.8	11.1	7.1	15.3	13.7	8.6
Diets												
Control	106^{c}	109^{c}	328^{b}	543^{c}	133^{b}	179^{b}	460^{b}	771^{b}	800^{c}	587^{bc}	714	704
Torula yeast RNA	114^{a}	123^{a}	345^{a}	582^{a}	136^{a}	190^{a}	482^{a}	808^{a}	834^{a}	622^{a}	717	718
Nupro®	113^{a}	119^{ab}	341^{a}	573^{ab}	138^{a}	186^{ab}	476^{ab}	800^{a}	818^{b}	616^{ab}	718	715
Glutamine	110^{b}	114^{bc}	338^{ab}	562^{b}	136^{a}	188^{a}	476^{ab}	800^{a}	809^{bc}	582^{c}	715	702
Pooled SEM	0.9	2.5	3.9	5.1	0.9	2.9	6.2	7.8	5.0	10.8	9.7	6.0
Challenges												
NC[2]	112^{a}	159^{a}	351^{a}	622^{a}	137^{a}	219^{a}	491^{a}	847^{a}	816	725^{a}	717	735^{a}
C[3]	109^{b}	72^{b}	325^{b}	507^{b}	134^{b}	151^{b}	455^{b}	740^{b}	814	476^{b}	714	684^{b}
Pooled SEM	0.7	1.8	2.8	3.6	0.7	2.0	4.4	5.5	3.6	7.6	6.8	4.3
Source of variation				----------		P		----------				
Diets	0.001	0.213	0.053	0.001	0.001	0.338	0.163	0.047	0.001	0.142	0.990	0.301
Challenges	0.030	0.001	0.001	0.001	0.007	0.001	0.001	0.001	0.907	0.001	0.748	0.001
Diets × Challenges	0.779	0.835	0.369	0.476	0.477	0.845	0.459	0.463	0.154	0.925	0.597	0.810

[a-d] Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means represent 9 replicates with 8 birds per replications and 72 birds per each treatment.

[2]NC; Non-challenged with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 4 d of age.

Table 4.4. Effects of dietary nucleotide (*Torula yeast* RNA, Nupro®, and glutamine) supplementation on nitrogen corrected apparent metabolizable energy (AME_N)[1] of experimental diets fed to broilers non-challenged or challenged with a high-dose Coccivac-B® vaccine at 4 d of age.

	AME_N	
Treatments	12 d	18 d
NC[2] + Control	2,724[a]	2,875[ab]
NC[2] + *Torula yeast* RNA	2,724[a]	2,957[a]
NC[2] + Nupro®	2,644[a]	2,811[bc]
NC[2] + glutamine	2,424[b]	2,567[e]
C[3] + Control	2,100[c]	2,877[ab]
C[3] + *Torula yeast* RNA	2,346[b]	2,812[bc]
C[3] + Nupro®	2,344[b]	2,648[de]
C[3] + glutamine	2,067[c]	2,727[cd]
Pooled SEM	69.6	46.2
Diets		
Control	2,394[b]	2,889[a]
Torula yeast RNA	2,546[a]	2,876[a]
Nupro®	2,503[ab]	2,729[b]
Glutamine	2,235[c]	2,647[b]
Pooled SEM	49.1	32.6
Challenges		
NC[2]	2,632[a]	2,807
C[3]	2,207[b]	2,762
Pooled SEM	34.7	23.0
Source of variation	---------- p ----------	
Diets	0.001	0.001
Challenges	0.001	0.271
Diets × Challenges	0.105	0.003

[a-e]Means within a column and parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means represent 9 replicates with 8 birds per replications.

[2]NC; Non-challenge with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 4 d of age.

CHAPTER 5

EFFECT OF DIETARY NUCLEOTIDE SUPPLEMENTATION ON PERFORMANCE AND DEVELOPMENT OF THE GASTROINTESTINAL TRACT OF BROILERS FED SEMI-PURIFIED DIETS AND CHALLENGED WITH COCCIVAC-B® [1]

[1] Jung, B. and A. B. Batal. To be submitted to *British Poultry Science*.

ABSTRACT

Two experiments were conducted to determine the effects of *Torula yeast* RNA as a dietary nucleotide supplementation on performance and intestinal tract development of birds fed semi-purified diets and challenged with Coccivac-B®. The experiments were a 2 × 2 factorial arrangement; two supplemental levels (0 and 0.25%) of *Torula yeast* RNA to semi-purified diets and either a challenge with Coccivac-B® or no challenge. One hundred and sixty eight 1-d-old Cobb 500 male broiler chicks were placed in battery brooders in six replicate pens containing seven chicks each. In experiment 1, birds were fed a casein-dextrose diet supplemented with 0 or 0.25% *Torula yeast* RNA for 7 d. In experiment 2, birds were fed a crystalline amino acid (AA) mixture-dextrose diet supplemented with 0 or 0.25% *Torula yeast* RNA for 13 d. Diets without nucleotide supplementation served as the controls in each experiment. At 5 d of age, one half of the birds from each dietary treatment in experiment 1 and 2 were inoculated with a high-dose (25 × higher than recommended vaccination dose) Coccivac-B® vaccine and remaining birds were inoculated with an equal volume of saline. *Experiment 1*: From 5 to 12 d of age, both the Coccivac-B® challenge and the supplementation of 0.25% *Torula yeast* RNA to the casein-dextrose diet did not significantly affect broiler performance. There were no significant differences in relative weight of small intestine and intestinal gross lesion scores of broilers due to the supplementation of 0.25% *Torula yeast* RNA and Coccivac-B® challenge. Villous height and crypt depth of broilers fed diets supplemented with 0.25% *Torula yeast* RNA were shorter than birds fed the casein-dextrose diet with no addition of the *Torula yeast* RNA but, the birds challenged with Coccivac-B® had longer villous than the non-challenged birds at 12 d of age. *Experiment 2*: From 5 to 18 d of age, the Coccivac-B® challenge and 0.25% *Torula yeast* RNA supplementation to the crystalline AA mixture-dextrose diet did not affect body weight (BW)

gain and feed:gain ratios of the broilers. However, from 9 to 12 d of age, 0.25% *Torula yeast* RNA supplementation diet increased feed intake as compared to the crystalline AA mixture-dextrose control diet. The supplementation of 0.25% *Torula yeast* RNA in the crystalline AA mixture-dextrose diet or challenging with the Coccivac-B® vaccine significantly increased jejuna relative weight as compared to birds fed the non-supplemented *Torula yeast* RNA crystalline AA mixture-dextrose diet or non-challenging. Also, birds challenged with Coccivac-B® had higher villous height and crypt depth at 12 d of age however, at 18 d of age the crypt depth was lower. In conclusion, birds fed semi-purified diets containing high levels of casein or crystalline amino acids mixture had very low feed intake and slow growth rate. The 25 × dose Coccivac-B® challenge did not have any additional effects on performance and the supplementation of 0.25% *Torula yeast* RNA to the semi-purified diets had no effects on performance of birds. Thus, nucleotide is not an essential nutrient without sufficient stress conditions.

(Key words: Semi-purified diets, Nucleotides, Broilers, Coccivac-B®)

INTRODUCTION

Nucleotides are the structural elements for nucleic acids including ribonucleic acids (RNAs) and deoxyribonucleic acids (DNAs). They are made of a nitrogenous base, pyrimidine or purine, a sugar (either ribose or deoxyribose), and one to three phosphate groups (Voet and Voet, 2004). Nucleotides are involved in a wide range of metabolic functions: energy metabolism (e.g. ATP), physiological mediators, and are components of coenzymes (Carver, 1999). They also have an effect on growth performance, development of intestine, intestinal morphology, enzyme concentrations, and immune system in animals (Carver and Walker, 1995; Uauy *et al*., 1990; Sanderson and He, 1994; Jung and Batal. 2011; Jyonouchi *et al*., 2001). Nucleotides are synthesized by a *de novo* pathway from amino acids and glucose. Nucleotides are also recycled through a salvage pathway in which the nucleotides are re-synthesized from intermediates generated during the breakdown of nucleotides (Carver, 1999). Nucleotides are not considered an essential nutrient because they can be synthesized by a *de novo* pathway or recycled from a salvage pathway thought to meet the requirements need by the body under unstressed conditions. However, dietary nucleotide may become essential when the endogenous supply is insufficient under certain conditions such as, rapid growth, recovery from injuries, certain disease states, environmental challenge, and reproduction (Yu, 1998; Carver, 1999).

Studies have investigated the effects of dietary nucleotide on performance and development of gastrointestinal tract of broilers or pigs in normal or certain stress conditions. Rutz *et al.* (2006) found that broilers fed a diet containing 2% yeast extract (Nupro®: contained 7% of total nucleotide content) had significantly better performance from 0 to 14 d of age as compared to birds fed an un-supplemented yeast extract diet. Jung and Batal (2011) found that the dietary nucleotide supplementation (0.25% *Torula yeast* RNA or 2% Nupro®) to a corn-

soybean meal based broiler diet significantly increased BW gain, feed efficiency, relative jejuna weight, and jejuna villous height of birds reared in certain stress conditions such as, previously used litter. Maribo (2003) reported that supplementation of 4.0% yeast extract product (Nupro®; contained 7% of free nucleotides) to pig diet improved performance as compared to the control diet with no supplementation of the yeast extract, when pigs were infected with an *Escherichia (E.) coli*. However, to our knowledge, no research has been conducting to evaluate performance, development of intestinal tract, and gross lesion scores of broilers fed semi-purified diet, which contains low levels of nucleotides. The objective of the research herein was to determine the effects of nucleotide (*Torula yeast* RNA*)* supplementation on performance and relative weight, morphology, and gross lesion scores in the small intestine of birds fed semi-purified diet containing high levels of casein or high levels of a crystalline amino acid mixture and challenged with Coccivac-B® vaccine.

MATERIALS AND METHODS

Birds and treatments

All experimental protocols were approved by the Animal Care and Use Committee at the University of Georgia. One-d-old Cobb 500 male broiler chicks were obtained from a commercial hatchery and placed into thermostatically controlled Petersime starter batteries (Petersime Incubator Co., Gettysburg, OH) with trough type feeders in an environmentally controlled building. Temperature and ventilation were monitored daily and adjusted as needed to maintain the temperatures recommended by the Cobb management guide (2004). At 5 d of age, after an overnight fast the chicks were weighed and assigned to four treatment groups so that mean initial weights were similar among all the treatment groups. In a 2 × 2 factorial

arrangement, one hundred and sixty eight birds were allotted to six replicate pens containing seven chicks each and were randomly assigned to two experimental diets (0 or 0.25% *Torula yeast* RNA supplemented diets) in the presence or absence of Coccivac-B® challenge at 5 d of age. Diets with no nucleotide supplementation served as the control in each experiment. Birds were fed a semi-purified diet containing high levels of casein (17%) with 0 or 0.25% *Torula yeast* RNA supplementation from 5 to 12 d of age in experiment (exp.) 1. In exp. 2 birds were fed a semi-purified diet containing high levels of a crystalline amino acid (AA) mixture (21.79%) with 0 or 0.25% *Torula yeast* RNA supplementation from 5 to 18 d of age. The total calculated nucleotide level in the two experimental diets in exp 1 was 0.35 and 0.51%, respectively (Table 5.1). On the other hand, in exp 2 the two experimental diets had 0.35 and 0.40% total nucleotide content, respectively Table 5.3). All treatment diets for exp. 1 and 2 were formulated to be isocaloric and isonitrogenous at 3,204 kcal ME/kg and 16.06% crude protein, respectively (Table 5.1 and 5.2). The corn starch-dextrose based diets with casein or crystalline amino acids (AA) mixture contain low levels of nucleotide so the nucleotides levels in the basal diet could be regulated (Uauy, 1990; Adjei *et al*., 1996). All birds had *ad libitum* access to feed and water and a 24 h lighting schedule. Calculated digestible lysine, total sulfur amino acid (TSAA) and threonine content in all the treatment diets were approximately 5% lower than Baker's (1997) recommendations whereas, calculated digestible amino acid ratios of all the treatment diets met and exceeded the Baker's (1997) recommendations (Table 6.3 and 6.4). Body weight (BW) and feed intake were evaluated on 8 and 12 d of age (3 and 7 d of post-challenge) in exp. 1 and evaluated on 8, 12, and 18 d of age (3, 7, and 13 d of post-challenge) in exp. 2. Feed efficiency (gain:feed) was calculated. At the occurrence of mortality, feed intake was adjusted based on bird days on feed.

Coccivac-B® challenge and inoculation dose

The recommended dosage level of Coccivac-B® vaccine (Intervet/Schering-Plough Animal health, USA) by manufacturer is approximately 1,395 oocysts per bird. Half of birds (84 birds) in exp. 1 and 2 from either the control or *Torula yeast* RNA supplemented diet group were challenged with a 25× dose of the Coccivac-B® vaccine which is approximately 34,875 oocysts per chick in 1 ml saline solution intubated directly into the crop via an oral gavages recommended by Dr. Mathis (a personal communication). The remaining birds (84 birds) received an equal volume of saline solution.

Analysis of small intestinal gross lesion scores

At 12 d of age (7 d post-challenge) one bird from each replicate pen (6 birds per treatment and total twenty four birds from each experiment) were euthanized by cervical dislocation. The gross lesion scoring system was based on a scale ranging from 0 to +4 with the level of infection increasing with number (Johnson and Reid, 1970). Generally a score of 0 = no gross lesions; 1 = very few scattered lesions and no thickening of the intestinal walls; 2 = a greater number of spread lesions with noticeable blood in the intestinal contents, and intestinal wall is somewhat thickened; 3 = lesions extensively developed with coalescence and some thickening of the intestinal walls; and 4 = extensive coalescence of lesions with thickening of the wall, bloody intestinal contents and dead birds scored as 4 (Johnson and Reid, 1970).

Analysis of relative weight and morphology of small intestine

One bird from each replicate pen, total of six birds from each treatment and total twenty four birds at each sampling time were randomly selected and euthanized on 8 and 12 d of age for

exp. 1 and on 8, 12, and 18 d of age for exp. 2 for analysis of the intestinal relative weight. Birds were weighed and then euthanized by cervical dislocation. The abdominal cavity was opened by mid-line incision and the small intestine was removed and divided into three segments: duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducks to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction). Any intestinal contents were removed from intestinal segments by flushing with a 0.9% saline solution, and the relative weight (g/100g of BW) of the empty small intestine was recorded (Miles *et al*., 2006).

For intestinal morphology analysis, one broiler chick from each replicate pen (six birds per treatment and total twenty four birds per each sampling time measured) was euthanized by cervical dislocation and a sample of the jejunum was taken at 12 d of age (7 d post-challenge) for exp. 1 and at 12 and 18 d of age (7 and 13 d post-challenge) for exp. 2. After emptying and flushing the jejunum with a 0.9% saline solution, approximately 5 cm of the middle portion of the jejunum was excised with surgical blade and fixed in 10% formalin solution until analysis. Three cross sections from each sample of 10% formalin-fixed, paraffin-embedded jejuna segments were sectioned at 3μm and stained with hematoxyline and eosin stain (Uni *et al*., 1995). A total of three, intact, well-oriented villi were selected with three replicates from each jejuna cross section (nine measurements for each jejuna sample with fifty four measurements per treatment). Intestinal epithelial villous height was measured from the tip of the villous to basement membrane of the villous, and crypt depth was measured from the villous bottom to the crypt. Villous height and crypt depth ratio was calculated. Images were taken by computer-aided light microscopy (10 × magnification of the objective lens and 1 × magnification of the adapter lens) and morphological indices were analyzed using Image pro plus version 3.0 image software.

Statistical analysis

All data were subjected to ANOVA procedure for completely randomized design (Steel and Torrie, 1980) using the general linear model procedure (PROC GLM) of SAS® software (SAS Institute, 2005). Statistical significance of differences among treatments was assessed using the Duncan's new multiple range test. All data were also analyzed by ANOVA to determine significance of main effects (diets and Coccivac-B® challenge) and interactions (diets × Coccivac-B® challenge). A probability level of $P < 0.05$ was used to determine statistical significance of differences among the treatments.

RESULTS AND DISCUSSION

Experiment 1: The results of supplementing the diet with nucleotides (0.25% *Torula yeast* RNA) on body weight (BW) gain, feed intake, and feed efficiency (gain:feed) of broilers fed a casein-dextrose diet and challenged with Coccivac-B® in experiment (exp) 1 are presented in Table 5.5. The supplementation of 0.25% *Torula yeast* RNA to a casein-dextrose diet did not affect BW gain and gain:feed at all periods measured while, the 0.25% *Torula yeast* RNA supplementation significantly decreased feed intake of birds regardless of the Coccivac-B® challenge from 9 to 12 d of age (4 to 7 d post-challenge). The Coccivac-B® challenge (25× of a dosage volume) did not affect BW gain, feed intake and feed efficiency significantly, but feed efficiency did numerically decrease from 9 to 12 d and 5 to 12 d of age. *Experiment 2*: The results of supplementing nucleotides (0.25% *Torula yeast* RNA) on body weight (BW) gain, feed intake, and feed efficiency (gain:feed) of broilers fed a crystalline amino acid (AA) mixture-dextrose diet and challenged with a Coccivac-B® in exp 2 are shown in Table 5.6. The Coccivac-B® challenge (25× of a dosage volume) and 0.25% *Torula yeast* RNA supplementation to the

crystalline AA mixture-dextrose diet did not affect BW gain and feed efficiency at all periods measured. However, from 9 to 12 d of age, the birds fed the crystalline AA mixture-dextrose diet supplemented with 0.25% *Torula yeast* RNA had increased feed intake as compared to the birds fed the crystalline AA mixture-dextrose control diet that was not added *Torula yeast* RNA. The birds challenged with Coccivac-B® had decreased feed intake as compared to the non-challenged birds from 9 to 12 d of age (4 to 7 d post-challenge). Thus, the supplementation of 0.25% *Torula yeast* RNA did not affect performance of broiler both challenged with the 25 × dose Coccivac-B® vaccine and non-challenged. This result disagree with the results of Jung and Batal(2011), who observed an improved performance of birds given nucleotide products (*Torula yeast* RNA; containing 62% total nucleotide content and Nupro®; containing 7% total nucleotide content) and a high-dose Coccivac-B® vaccine challenge. However, the birds in the previous study reported by Jung and Batal (2011) were fed a corn-soybean meal basis and they grew and fed diets normally as compared to the recommendations (Cobb management guide, 2008). The birds were also challenged with a high-dose (50 ×) Coccivac-B® vaccine, which caused decreased performance (Jung, 2011). On the other hand, the 25 × dose Coccivac-B® challenge did not have any additional effects on performance in birds in the current experiments. The averaged BW and feed intake of the birds at 12 d of age was 190g and 230g, respectively in exp. 1 and 170g and 215g, respectively in exp. 2 and the averaged BW and feed intake of bird at 18 d of age was 270g and 420g, respectively in exp. 2. The averaged BW and feed intake of birds based on Cobb 500 guide (2008) was 372g and 414g, respectively and 667g and 854g, respectively at the same days of age. Therefore, the reason for no performance responses of birds fed semi-purified diets supplemented with *Torula yeast* RNA or challenged with Coccivac-B® vaccine may be due to the slow growth rate and low feed intake of the birds. Insufficient stress conditions in the two

current experiments may also be one of the possible causes of the absence of differences on performance due to the supplementation of nucleotides. Pelicia *et al.,* (2009) also did not observe any performance improvement in birds fed a diet supplemented with up to 0.07% nucleotide-rich product (containing 22.5% Total nucleotides content) as compared to those fed a diet non-supplemented with nucleotides product when birds are reared on new litter and under strict health control. Cruickshank (2002) and Maribo (2003) recommended that stress conditions, such as environmental changes or challenge and infection stress is required to see beneficial effects on performance due to the supplementation of nucleotides-rich product in broilers or pigs.

Experiment 1: No significant difference in the small intestinal relative weight on 8 and 12 d of age (3 and 7 d post-challenge, respectively) was observed due to the supplementation of 0.25% *Torula yeast* RNA and with a challenge with Coccivac-B® vaccine (Table 5.7.). *Experiment 2*: There was no difference on relative weight of the duodenum on 8, 12, and 18 d of age (3, 7, and 13 d post-challenge, respectively) due to the supplementation of 0.25% *Torula yeast* RNA (Table 5.8.). However, on 12 d of age (7 d post-challenge) when the non-challenged or challenged birds were fed the crystalline AA mixture-dextrose diet supplemented with 0.25% *Torula yeast* RNA, the jejuna relative weight was increased as compared to the birds fed diets. Also, the supplementation of 0.25% *Torula yeast* RNA increased ($P < 0.057$) ileum relative weight of birds challenged with Coccivac-B® at 18 d of age (13 d post-challenge). The Coccivac-B® challenge did not affect relative duodenum and ileum weight at 8, 12, and 18 d of age but, this challenge significantly increased relative jejuna weight at 12 d of age. Similarly, researcher has reported that when birds were infected with *Coccidiosis*, it lead to increased relative small intestine weight (Yvore, 1978 ; Allen, 1984). In detail, Allen (1984) reported that increased fresh weights of jejunum at 6 d post-infection which is corresponds to a period of maximum

oocyst shedding were observed due to a *E. acervulina* (2×10^6 sporulated oocysts /bird) infection. The increase in weights of jejunum may be associated with changed morphology such as, villi height and width, mucosal weight and thickness due to cellular infiltration induced by parasitic infection (Allen, 1987; Raines, 1989). In the current experiments, the birds challenged with Coccivac-B® had significantly longer villous in jejunum than the birds that were not challenged at 12 d of age (7 d post-challenge).

Experiment 1: The supplementation of 0.25% *Torula yeast* RNA to the casein-dextrose diet significantly decreased gross lesion scores in the jejunum of birds challenged with Coccivac-B® (Table 5.9). In the cecum the birds challenged with Coccivac-B® vaccine had increased gross lesion scores as compared to the non-challenged birds fed casein-dextrose diet while, the 0.25% *Torula yeast* RNA supplementation did not affect gross lesion scores. In the jejunum there is no effect on gross lesion scores due to the Coccivac- B® challenge but, the supplementation of 0.25% *Torula yeast* RNA to the casein-dextrose diet significantly decreased gross lesion scores. These two responses resulted in diets × Coccivac-B® challenges interactions for gross lesions scores in cecum ($P< 0.048$) and jejunum ($P< 0.010$). *Experiment 2*: The is no effect on gross lesion scores in the jejunum ileum, and cecum due to the addition of 0.25% *Torula yeast* RNA and Coccivac-B® vaccine challenge (Table 5.10). While, the Coccivac-B® challenge significantly increased gross lesion scores in the duodenum as compared to the non-challenged birds at 12 d of age (7 d post-challenge). The gross lesion scores in small intestine were changed due to the inoculation of *E. acervulina, E. tenella, or E. maxima* to broilers with positive correlation to inoculation dose; upward shift of lesions scores as increasing oocyst doses of *E. species* (Conway *et al.*, 1993). In the both current experiments the gross lesion scores in duodenum in birds challenged with Coccivac-B® were significantly increased as compared to the lesion scores

of non-challenged birds. The Coccivac-B® vaccine contains *E. acevulina, E. tenella, E. maxima, and E. mivatti.* This vaccine contains higher levels of *E. acevulina* which mainly causes histopathology changes in duodenum than the other *E. species* (Intervet[2]; Witlock and Ruff, 1977). Thus, the Coccivac-B® challenge may be more effective in duodenum in the current experiments.

Experiment 1: Villous height and crypt depth of broilers fed the casein-dextrose diet supplemented with 0.25% *Torula yeast* RNA were significantly shorter than those of the birds fed the casein control diet without the *Torula yeast* RNA supplementation regardless of challenge with Coccivac-B® vaccine (Table 5.11). While, the birds challenged with Coccivac-B® had significantly longer villous in jejunum than the birds that were not challenged on 12 d of age (7 d post-challenge) (Table 5.11). There were no effects on the villous height to crypt depth ratio due to the addition of 0.25% *Torula yeast* RNA and Coccivac-B® challenge. *Experiment 2:* The supplementation of 0.25% *Torula yeast* RNA to crystalline AA mixture-dextrose diets did not affect villous height, crypt depth, and villous height to crypt depth ratio of birds regardless of challenge with Coccivac-B® vaccine at 12 d and 18 d of age (7 and 13d post-challenge, respectively) (Table 5.12). The birds challenged with Coccivac-B® vaccine had significantly higher villous height and crypt depth but, smaller villous height to crypt depth ratio as compared to the non-challenged birds at 12 d of age (7 d post-challenge). However, at 18 d of age (13 d post-challenge) the challenging birds with Coccivac-B® vaccine had shorter villous height and lower villous height to crypt depth ratio and deeper crypt depth as compared to the non-challenged birds. When birds were not challenged the supplementation of 0.25% *Torula yeast* RNA to the crystalline AA mixture-dextrose diets significantly increased villous height and

[2] Intervet/Schering-Plough Animal Health, USA)

villous height to crypt depth ratio in jejunum as compared to the crystalline AA mixture-dextrose control diet at 12 d of age (7 d post-challenge). On the other hand, at 18 d of age when birds were challenged with Coccivac-B® the supplementation of 0.25% *Torula yeast* RNA to the diets increased crypt depth as compared to the crystalline AA mixture-dextrose control diet. The effects of *Coccidiosis* inoculation on intestinal morphology have been reported in several studies in broilers (Jung, 2011; Witlock and Ruff, 1977; Allen, 1987). For example, a high-dose (50 ×) Coccivac-B® challenge significantly increased villous height in jejunum and ileum at 13 d of age (9 d post-challenge) in broilers (Jung, 2011). Witlock and Ruff (1977) also reported that bird infected with *E. maxima* had longer jejuna villous and deeper crypt depth in acute phase of *Coccidiosis* (7 d post-infection). The results from our current two experiments agreed with aforementioned studies. Birds challenged with Coccivac-B® had significantly longer jejuna villous than the unchallenged birds at accurate phase of *Coccidiosis* (7 d post-challenge). Also, Coccivac-B® challenge significantly increased the crypt depth in small intestine at 12 d and 18 d of age (7 d and 14 d post-challenge, respectively) in exp. 2. This result was consistent to the result from a study that infected birds with *E. acervulina (*Allen, 1987). Increased villous height is indicative of increase surface, which may compensate damaged intestinal epithelial cells to absorb nutrients in the gut. Also, increased crypt depth indicate fast tissue turnover to permit renewal of the villous as needed in response to normal sloughing or inflammation from pathogens and high demands for epithelial tissue (Yason *et al.,* 1987). The effects of the supplementation of nucleotide products on intestinal morphology have been reported in several studies (Jung and Batal, 2011; Martinez-Puig *et al.,* 2007). Dietary nucleotide (0.25% *Torula yeast* RNA (containing 62% total nucleotide content and 2% Nupro® (contained 7% total nucleotide content) supplementation increased jejuna villous height in birds reared in

environmental stress conditions (previously used litter) at 14 d of age (Jung and Batal, 2011). In addition, in a swine study, early-weaned piglets fed a diet supplemented with 0.1% or 0.2% of commercial nucleotide product (Nucleoforce Piglets®; contained 25% free nucleotide content) had higher villous in ileum when compared to piglets fed control diet with no nucleotide supplementation (Martinez-Puig *et al*., 2007). In the current exp. 2, when birds were not challenged with Coccivac-B®, the supplementation of 0.25% *Torula yeast* RNA in the crystalline AA mixture-dextrose diet increased villous height as compared to the crystalline AA mixture-dextrose control diets at 12 d of age (7 d post-challenge). However, villous height of birds fed casein-dextrose diet supplemented with 0.25% *Torula yeast* RNA were significantly shorter than the birds fed the casein diet without the *Torula yeast* RNA supplementation when the birds are both challenged and non-challenged in exp. 1.

Challenging birds with Coccivac-B® vaccine fed semi-purified diets contained high levels of casein or a crystalline amino acids mixture supplemented with the supplementation of the 0.25% *Torula yeast* RNA did not affect BW gain, feed intake, and feed efficiency. Lack of responses may be due to slow growing rate and low feed intake of birds fed semi-purified diets. Also, the 25 × dose Coccivac-B® challenge did not have any additional effect on performance. It is concluded that nucleotides may not be an essential nutrient without sufficient stress conditions in birds

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Table 5.1. Composition and total nucleotide content of the dietary treatments[1] from 5 to 12 d of age (as-fed basis). Experiment 1.

Ingredient, %	Casein (T1 and T3)	Casein + *Torula yeast* RNA (T2 and T4)
Corn starch	16.00	16.00
Dextrose	50.36	50.36
Solkafloc	5.00	4.75
Casein[2]	17.00	17.00
Poultry fat	3.00	3.00
Dicalcium phosphate	1.50	1.50
Limestone	1.22	1.22
Salt	0.02	0.02
Vitamin Mix[3]	0.50	0.50
Mineral mix[4]	0.16	0.16
$NaHCO_3$	2.00	2.00
KCl	1.20	1.20
MgO	0.20	0.20
DL-Methionine	0.38	0.38
L-Threonine	0.17	0.17
L-Phenylalanine	0.54	0.54
L-Arginine	0.75	0.75
Torula yeast RNA[5]	-	0.25
Total	100.0	100.0
Contents by calculation		
ME, Kcal/kg	3,204	3,204
Crude protein, %	16.06	16.06
Calcium, %	0.90	0.90
Avail. phosphorus, %	0.45	0.45
Calculated total nucleotide content[6]	0.35	0.51

[1] Non-challenged group: T1 and T2; Challenged group with Coccivac-B® vaccine at 5 d of age: T3 and T4.
[2]International Ingredient Corporation, Saint Louis, MO.
[3]Vitamin mix provided the following (per kg of diet) : Thiamin mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12mg; vitamin B_{12}(cobalamin), 12.0µg; pyridoxine HCL, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5µg; trans-retinyl acetate, 1,892µg; all-rac α tocopheryl acetate, 11mg; ethozyquin, 125mg.
[4]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; idodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.
[5]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.
[6] Ingredient samples were sent to All-tech®, Nicholasville, KY, 40356 for nucleotide quantification.

Table 5.2. Calculated digestible amino acid and ratios of the dietary treatments[1] (as-fed basis).

Experiment 1.

Digestible amino acids	Casein (T1 and T3)	Casein + *Torula yeast* RNA (T2 and T4)
Arginine (1.18)[2]	1.22	1.22
Histidine (0.39)	0.50	0.50
Isoleucine (0.75)	0.91	0.92
Leucine (1.22)	1.66	1.66
Lysine (1.12)	1.07	1.07
TSAA (0.81)	0.77	0.77
Phe+Tyr (1.18)	1.61	1.62
Threonine (0.75)	0.70	0.70
Valine (0.86)	0.99	1.00
Calculated digestible amino acid ratios		
Arg/lys[3] (105) [4]	116	116
Met/lys (36)	73	73
TSAA/lys (72)	78	78
Trp/lys (16)	16	16
His/lys (35)	37	37
Leu/lys (109)	130	130
Ile/lys (67)	85	85
Phe + Tyr/lys (105)	115	115
Thr/lys (67)	71	71
Val/lys (77)	102	102

[1]Non-challenged group: T1 and T2; Challenged group with Coccivac-B® vaccine at 5 d of age: T3 and T4.

[2]Digestible amino acid recommendations for starter period in broilers (Baker, 1997).

[3]Calculation: Calculated digestible amino acid concentration / calculated digestible lysine concentration × 100.

[4]Ideal amino acid ratio recommendations for the starter period in broilers (Baker, 1997).

Table 5.3. Composition and total nucleotide content of the dietary treatments[1] from 5 to 18 d of age (as-fed basis). Experiment 2.

Ingredients, %	Crystalline amino acids (AAs) (T1 and T3)	Crystalline AAs + *Torula yeast* RNA (T2 and T4)
Corn starch	15.55	15.55
Dextrose	50.00	50.00
Solkafloc	5.00	4.75
Crystalline amino acids mixture[2]	21.79	21.79
Poultry fat	0.20	0.20
Dicalcium phosphate	2.40	2.40
Limestone	0.98	0.98
Salt	0.02	0.02
Vitamin Mix[3]	0.50	0.50
Mineral mix[4]	0.16	0.16
$NaHCO_3$	2.00	2.00
KCl	1.20	1.20
MgO	0.20	0.20
Torula yeast RNA[5]	-	0.25
Total	100.0	100.0
Content by calculation		
ME, Kcal/kg	3,204	3,204
Crude protein, %	16.06	16.06
Calcium, %	0.90	0.90
Avail. phosphorus, %	0.45	0.45
Calculated total nucleotide content[6]	0.35	0.40

[1]Non-challenged group: T1and T2; Challenged group with Coccivac-B® vaccine at 5 d of age: T3 and T4.
[2]Crystalline Amino acids mixture (% of the diet) : L-Lysine-HCl, 1.447; L-arginine, 1.320; L-Histidine-HCl-H20, 0.581; DL-Methionine, 0.838; L-Cystine, 0.050; L-Phenylalanine, 0.700; L-Tyrosine, 0.610; L-Threonine, 0.822; L-Leucine, 1.480; L-Isoleucine, 0.970; L-Valine, 1.160; L-Tryptophan, 0.183; L-Glycine, 0.609; L-Proline, 0.406; L-Glutamic acid, 10.610.
[3]Vitamin mix provided the following (per kg of diet) : Thiamin mononitrate, 2.4mg; nicotinic acid, 44mg; D-Ca pantothenate, 12mg; vitamin B_{12}(cobalamin), 12.0µg; pyridoxine HCL, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfate complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5µg; trans-retinyl acetate, 1,892µg; all-rac α tocopheryl acetate, 11mg; ethozyquin, 125mg.
[4]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \cdot H_2O$), 60mg; iron ($FeSO_4 \cdot 7H_2O$), 30mg; zinc (ZnO), 50mg; copper ($CuSO_4 \cdot 5H_2O$), 5mg; idodine (ethylene diamine dihydroiodide), 0.15mg; selenium ($NaSeO_3$), 0.3mg.
[5]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.
[6]Ingredient samples were sent to All-tech®, Nicholasville, KY, 40356 for nucleotide quantification.

Table 5.4. Calculated digestible amino acid and ratios of the dietary treatments[1] (as-fed basis).

Experiment 2.

Digestible amino acids	Amino acids (T1 and T3)	Amino acids + *Torula yeast* RNA (T2 and T4)
Arginine (1.18)[2]	1.19	1.23
Histidine (0.39)	0.50	0.50
Isoleucine (0.75)	0.86	0.92
Leucine (1.22)	1.64	1.66
Lysine (1.12)	1.07	1.07
TSAA (0.81)	0.77	0.77
Phe+Tyr (1.18)	1.59	1.62
Threonine (0.75)	0.70	0.70
Valine (0.86)	0.99	1.00
Calculated digestible amino acid ratios		
Arg/lys[3] (105)[4]	116	116
Met/lys (36)	73	73
TSAA/lys (72)	78	78
Trp/lys (16)	16	16
His/lys (35)	38	38
Leu/lys (109)	130	130
Ile/lys (67)	85	85
Phe + Tyr/lys (105)	115	115
Thr/lys (67)	71	71
Val/lys (77)	102	102

[1]Non-challenged group: T1and T2; Challenged group with Coccivac-B® vaccine at 5 d of age: T3 and T4.

[2]Digestible amino acid recommendations for the starter period in broilers (Baker, 1997).

[3]Calculation: Calculated digestible amino acid concentration / calculated digestible lysine concentration × 100.

[4]Ideal amino acid ratio recommendations for the starter period in broilers (Baker, 1997).

[5]Ingredient samples were sent to All-tech®, Nicholasville, KY, 40356 for nucleotide quantification.

Table 5.5. Effects of a dietary nucleotide (*Torula yeast* RNA) supplementation on BW gain (g/chick)[1], feed intake[1] (g/chick), and feed efficiency[1] (gain:feed, g:kg) of broilers fed casein-dextrose diets and challenged with Coccivac-B® vaccine at 5 d of age. Experiment 1.

	BW gain (g/chick)			Feed intake (g/chick)			gain:feed, g:kg		
Treatments	5 to 8 d	9 to 12 d	5 to 12 d	5 to 8 d	9 to 12 d	5 to 12 d	5 to 8 d	9 to 12 d	5 to 12 d
NC[2] + Casein	38	74	111	46	91	138	814	802	807
NC[2]+ Casein + *Torula yeast* RNA	42	73	114	50	84	134	839	861	853
C[3] + Casein	38	70	108	49	95	144	790	736	752
C[3] + Casein + *Torula yeast* RNA	41	66	107	47	84	131	864	795	817
Pooled SEM	2.3	4.8	5.8	2.0	3.4	4.4	38.9	44.4	31.5
Diets									
Control	38	72	110	47	93[a]	141	802	769	780
Torula yeast RNA	41	69	111	49	84[b]	133	852	828	835
Pooled SEM	1.6	3.4	4.1	1.4	2.4	3.1	27.4	31.4	22.3
Challenges									
NC[2]	40	73	113	48	88	136	827	832	830
C[3]	40	68	108	48	89	137	827	766	784
Pooled SEM	1.6	3.4	4.1	1.4	2.4	3.1	27.5	31.4	22.3
Source of variation			----------		P	----------			
Diets	0.140	0.603	0.863	0.601	0.015	0.080	0.215	0.199	0.095
Challenges	0.943	0.322	0.398	0.972	0.644	0.729	0.991	0.152	0.161
Diets × Challenges	0.762	0.760	0.711	0.198	0.581	0.310	0.538	0.999	0.772

[a,b] Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means represent 6 replicates with 7 birds per replications and 42 birds per each treatment.

[2]NC; Non-challenged with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 5 d of age.

Table 5.6. Effects of a dietary nucleotide (*Torula yeast* RNA) supplementation on BW gain (g/chick)[1], feed intake[1] (g/chick), and feed efficiency (gain:feed, g:kg)[1] of broilers fed crystalline amino acids (AAs) mixture-dextrose diets and challenged with Coccivac-B® vaccine. Experiment 2.

	BW gain (g chick)			Feed intake (g/chick)			gain:feed, g:kg		
Treatments	5 to 8 d	9 to 12 d	5 to 18 d	5 to 8 d	9 to 12 d	5 to 18 d	5 to 8 d	9 to 12 d	5 to 18 d
NC[2]+ AAs	32	56	182	48	83[ab]	317	660	670	573
NC[2] + AAs + *Torula yeast* RNA	30	57	180	46	93[a]	331	638	613	543
C[3] + AAs	28	53	196	45	79[b]	332	634	670	594
C[3] + AAs + *Torula yeast* RNA	31	55	183	51	84[ab]	349	610	658	528
Pooled SEM	1.2	3.7	10.7	2.6	3.2	14.8	32.2	43.1	23.9
Diets									
Control	30	55	189	47	81[b]	324	647	670	583
Torula yeast RNA	30	56	182	49	88[a]	340	624	635	536
Pooled SEM	0.9	2.6	7.6	1.8	2.2	10.5	22.7	30.4	16.9
Challenges									
NC[2]	31	57	181	47	88[a]	324	649	642	558
C[3]	29	54	189	48	82[b]	340	622	664	561
Pooled SEM	0.9	2.6	7.6	1.8	2.2	10.5	22.7	30.4	16.9
Source of variation									
Diets	0.965	0.743	0.515	0.482	0.042	0.303	0.486	0.428	0.059
Challenges	0.274	0.480	0.446	0.780	0.048	0.280	0.413	0.614	0.914
Diets × Challenges	0.065	0.965	0.599	0.125	0.419	0.903	0.973	0.602	0.459

[a,b]Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means represent 6 replicates with 7 birds per replications and 42 birds per each treatment.

[2]NC; Non-challenged with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 5 d of age.

Table 5.7. Effects of a dietary nucleotide (*Torula yeast* RNA) supplementation on small intestine relative weight (g/kg of BW)[1] of broilers fed casein-dextrose diets and challenged with Coccivac-B® vaccine at 5 d of age. Experiment 1.

	8 d of age			12 d of age		
Treatments	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
NC[2]+ Casein	25.2	35.9	28.1	16.0	25.5	19.4
NC[2]+ Casein + *Torula yeast* RNA	22.2	34.3	26.4	18.2	27.7	21.7
C[3] + Casein	22.5	33.7	25.0	17.1	26.9	20.4
C[3]+ Casein + Torula yeast RNA	21.0	31.4	26.5	18.8	29.4	21.6
Pooled SEM	1.33	2.32	2.27	1.27	1.77	1.90
Diets						
Control	23.9	34.8	26.5	16.5	26.2	19.9
Torula yeast RNA	21.6	32.8	26.5	18.5	28.5	21.7
Pooled SEM	0.94	1.64	1.61	0.90	1.26	1.34
Challenges						
NC[2]	23.7	35.1	27.2	17.1	26.6	20.6
C[3]	21.7	32.6	25.8	17.9	28.1	21.0
Pooled SEM	0.94	1.64	1.61	0.90	1.26	1.34
Source of variation			---------- P ----------			
Diets	0.104	0.402	0.988	0.136	0.209	0.361
Challenges	0.155	0.287	0.521	0.518	0.406	0.832
Diets × Challenges	0.605	0.865	0.485	0.835	0.923	0.798

[1]Means of 6 samples per treatment (1 bird per replication, total 24 birds) at each sampling time.

[2]NC; Non-challenged with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 5 d of age.

Table 5.8. Effects of a dietary nucleotide (*Torula yeast* RNA) supplementation on small intestine relative weight (g/kg of BW)[1] of broilers fed crystalline amino acids (AAs) mixture-dextrose diets and challenged with Coccivac-B® vaccine at 5 d of age. Experiment 2.

Treatments	8 d of age			12 d of age			18 d of age		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
NC[2]+ AAs	22.1	30.8	20.5	19.5	24.1[b]	19.2	14.4	20.2	11.8[ab]
NC[2] + AAs + *Torula yeast* RNA	21.3	35.6	24.8	19.1	28.7[ab]	19.4	14.1	22.3	11.7[ab]
C[3] + AAs	23.8	34.8	24.9	18.3	28.4[ab]	17.8	12.5	19.4	10.8[b]
C[3] + AAs + *Torula yeast* RNA	20.9	31.0	24.6	19.8	32.2[a]	18.7	15.0	20.8	13.6[a]
Pooled SEM	1.37	2.41	1.59	1.14	1.75	1.74	0.86	1.09	0.67
Diets									
Control	22.9	32.8	22.7	18.9	26.2[b]	18.5	13.4	19.7	11.3
Torula yeast RNA	21.1	33.3	24.7	19.5	30.4[a]	19.1	14.5	21.4	12.6
Pooled SEM	0.97	1.32	1.12	0.81	1.24	1.23	0.61	0.77	0.47
Challenges									
NC[2]	21.7	32.2	22.7	19.3	26.4[b]	19.3	14.2	21.3	11.8
C[3]	22.3	33.0	24.7	19.1	30.3[a]	18.3	13.7	20.0	12.2
Pooled SEM	0.97	1.32	1.12	0.81	1.24	1.23	0.61	0.76	0.47
Source of variation	----------			P		----------			
Diets	0.187	0.798	0.229	0.618	0.026	0.749	0.218	0.139	0.057
Challenges	0.650	0.901	0.205	0.828	0.039	0.570	0.547	0.263	0.544
Diets × Challenges	0.467	0.033	0.156	0.446	0.825	0.850	0.117	0.671	0.047

[a,b]Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means of 6 samples per treatment (1 bird per replication, total 24 birds) at each sampling time.

[2]NC; Non-challenged with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 5 d of age.

Table 5.9. Effects of a dietary nucleotide (*Torula yeast* RNA) supplementation on the gross lesion scores[1] of the small intestine of broilers fed casein-dextrose diet and challenged with Coccivac-B® vaccine at 5 d of age. Experiment 1.

	12 d of age			
Treatments	Duodenum	Jejunum	Ileum	Cecum
NC[2] + Casein	0.00	0.17[ab]	0.00	0.17[b]
NC[2]+ Casein + *Torula yeast* RNA	0.00	0.50[ab]	0.00	0.67[a]
C[3] + Casein	0.33	0.67[a]	0.17	0.17[b]
C[3] + Casein + *Torula yeast* RNA	0.33	0.00[b]	0.00	0.00[b]
Pooled SEM	0.149	0.175	0.083	0.158
Diets				
Control	0.17	0.42	0.08	0.08
Torula yeast RNA	0.17	0.25	0.00	0.00
Pooled SEM	0.105	0.124	0.059	0.059
Challenges				
NC[2]	0.00[b]	0.33	0.00	0.42[a]
C[3]	0.33[a]	0.33	0.08	0.08[b]
Pooled SEM	0.105	0.124	0.059	0.112
Source of variation		---------- P ----------		
Diets	1.000	0.352	0.329	0.304
Challenges	0.037	1.000	0.329	0.048
Diets × Challenges	1.000	0.010	0.329	0.048

[a,b] Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means of 6 samples per treatment (1 bird per replication, total 24 birds).

[2]NC; Non-challenged with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 5 d of age.

Table 5.10. Effects of a dietary nucleotide (*Torula yeast* RNA) supplementation on the gross lesion scores[1] small intestine of broilers fed crystalline amino acids (AAs) mixture-dextrose diets and challenged with Coccivac-B® vaccine at 5 d of age. Experiment 2.

	12 d of age			
Treatments	Duodenum	Jejunum	Ileum	Cecum
NC[2] + AAs	0.00[b]	0.17	0.00	0.17
NC[2] + AAs + *Torula yeast* RNA	0.00[b]	0.00	0.17	0.17
C[3] + AAs	0.50[ab]	0.33	0.00	0.00
C[3] + AAs + *Torula yeast* RNA	0.83[a]	0.33	0.00	0.00
Pooled SEM	0.190	0.171	0.083	0.118
Diets				
Control	0.25	0.25	0.00	0.08
Torula yeast RNA	0.42	0.17	0.08	0.08
Pooled SEM	0.134	0.121	0.059	0.083
Challenges				
NC[2]	0.00[b]	0.08	0.08	0.17
C[3]	0.67[a]	0.33	0.00	0.00
Pooled SEM	0.134	0.121	0.059	0.083
Source of variation		---------- P ----------		
Diets	0.391	0.631	0.329	1.000
Challenges	0.002	0.159	0.329	0.173
Diets × Challenges	0.391	0.631	0.329	1.000

[a,b]Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Means of 6 samples per treatment (1 bird per replication, total 24 birds).

[2]NC; Non-challenged with Coccivac-B® vaccine.

[3]C; Challenged with Coccivac-B® vaccine at 5 d of age.

Table 5.11. Effects of a dietary nucleotide (*Torula yeast* RNA)[1] supplementation on jejuna morphology[2] of broilers fed casein-dextrose diets and challenged with Coccivac-B® vaccine at 5 d of age. Experiment 1.

	12 d of age		
Treatments	Villous height, µm	Crypt depth, µm	Villous height : crypt depth ratios
NC[3] + Casein	677[a]	147[a]	4.9
NC[3]+ Casein + *Torula yeast* RNA	615[b]	127[b]	5.1
C[4] + Casein	703[a]	146[a]	5.1
C[4] + Casein + *Torula yeast* RNA	669[a]	130[b]	5.3
Pooled SEM	13.6	4.7	0.21
Diets			
Control	690[a]	147[a]	5.1
Torula yeast RNA	639[b]	128[b]	5.2
Pooled SEM	9.6	3.3	0.15
Challenges			
NC[3]	647[b]	137	5.0
C[4]	687[a]	139	5.2
Pooled SEM	9.6	3.3	0.15
Source of variation	----------	P	----------
Diet	0.003	0.800	0.439
Challenges	0.001	0.001	0.441
Diets × Challenges	0.290	0.777	0.957

[a,b]Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[2]Means of 54 measurements per treatment (9 measurements for each jejuna sample × 6 birds per treatment, total 24 birds).

[3]NC; Non-challenged with Coccivac-B® vaccine.

[4]C; Challenged with Coccivac-B® vaccine at 5 d of age.

Table 5.12. Effects of a dietary nucleotide (*Torula yeast* RNA)[1] supplementation on jejuna morphology[2] of broilers fed crystalline amino acid AAs mixture-dextrose diets and challenged with Coccivac-B® vaccine at 5 d of age. Experiment 2.

Treatments	12 d of age, µm			18 d of age, µm		
	Villous height	Crypt depth	Villous height : crypt depth ratios	Villous height, µm	Crypt depth, µm	Villous height : crypt depth ratios
NC[3] + Crystalline AAs	641[b]	115[b]	5.8[b]	875	84[b]	10.9[a]
NC[3]+ Crystalline AAs + *Torula yeast* RNA [1]	691[a]	114[b]	6.9[a]	878	84[b]	10.8[ab]
C[4] + Crystalline AAs	712[a]	133[a]	5.7[b]	845	90[b]	9.8[bc]
C[4] + Crystalline AAs + *Torula yeast* RNA	694[a]	129[a]	5.6[b]	851	99[a]	9.2[c]
Pooled SEM	14.9	4.6	0.27	13.9	3.2	0.37
Diets						
Control	678	125	5.8	859	87	10.3
Torula yeast RNA	693	123	6.1	865	91	10.1
Pooled SEM	10.5	3.2	0.19	9.8	2.3	0.3
Challenges						
NC[3]	664[b]	114[b]	6.3[a]	877[a]	84[b]	10.9[a]
C[4]	703[a]	131[a]	5.7[b]	848[b]	94[a]	9.5[b]
Pooled SEM	10.5	3.2	0.19	9.8	2.2	0.3
Source of variation		----------		P	----------	
Diets	0.150	0.001	0.012	0.041	0.001	0.001
Challenges	0.297	0.515	0.113	0.749	0.167	0.338
Diets × Challenges	0.024	0.746	0.026	0.907	0.149	0.463

[a-c]Means within a column and within a parameter without a common superscript differ significantly ($P < 0.05$).

[1]Ribonucleic acid from *Torula yeast* Type VI, R6625, Sigma-Aldrich INC, Saint Louis, MO.

[2]Means of 54 measurements per treatment (9 measurements for each jejuna sample × 6 birds per treatment, total 24 birds).

[3]NC; Non-challenged with Coccivac-B® vaccine.

[4]C; Challenged with Coccivac-B® vaccine at 5 d of age.

CHAPTER 6

CONCLUSIONS

Work by other researchers has shown that nucleotide production by *de novo* or the salvage pathway may not be sufficient to supply adequate levels of nucleotides for biologically active cells such as rapidly proliferating epithelium cells in the intestine and lymphocytes in bone marrow. Thus, an exogenous supplementation of nucleotides through the diet may be essential to maintain growth, development of the gastrointestinal tract, and modulate the immune system in animals. The use of a dietary nucleotide supplementation may be of particular importance in biological or environmental stress situations such as malnutrition, a disease infection, or environmental stress in which the rate of cell proliferation in the intestine or activation of immune system are elevated. However, little is known about the needs for dietary nucleotides and their role on performance, intestinal morphology, gastrointestinal tract development, and immune system of birds that are under stress conditions; previously used litter, *Coccidiosis* challenge alone or combined with an increased stocking density in birds. Therefore, the overall objective was to determine the effect of dietary nucleotide products (*Torula yeast* RNA and Nupro®) or a nucleotide precursor (glutamine) on performance, physical and morphological development of gastrointestinal tract, lesions score, serum citrulline level, and nutrient utilization of broilers fed a corn-soybean based diet or a corn starch-dextrose based diet under normal or certain stress conditions such as, previously used litter, *Coccidiosis* challenge alone, or combined with an increased stocking density. The supplementation of dietary nucleotides to a corn-soybean based or semi-purified based diet did not have an effect on performance or development of

gastrointestinal tract of broilers under normal or without sufficient stress conditions. However, supplementing the diets with dietary nucleotides improved performance of birds reared in previously used litter or the increased stocking density. The supplementation of dietary nucleotides was also effective at alleviating some negative effects of a *Coccidiosis* infection on performance and AME_N value of diets. In conclusion, the finding from the work reported herein indicates that nucleotides are not an essential nutrient under normal conditions. However, dietary nucleotide supplementation may be important to maintain maximum growth performance and AME_N value of diets, when birds are exposed to stress conditions, such as previously used litter, *Coccidiosis* challenge alone, or combined with the increased stocking density.

APPENDICES

APPENDIX A: ANALYSIS OF NUCLEOTIDE LEVEL IN FEED INGREDIENTS

The value for nucleotide content in feed ingredients is highly limited. A test was conducted to analyze the total nucleotide content or total ribonucleic acid (RNA) content in select feed ingredients. The feed ingredients were sent to two commercial laboratories, Chemoforma Ltd. and All-tech®, for total nucleotide or total RNA quantification analysis, respectively. The total nucleotide content in corn was 0.17% and total RNA content in corn ranged from 0.47 to 1.08% with a mean of 0.80%. Soybean meal contained 0.79% total nucleotide and ranged from 1.31 to 1.62% with a mean of 1.42% total RNA content. The total nucleotide content in a commercially available product (Nupro®) was 3.37% and averaged 7.81% total RNA content. *Torula yeast* RNA contained 61.7% total nucleotide content and ranged between 44.3 and 82.0% with a mean of 65.8% total RNA content. The total RNA content in distillers dried grain with solubles (DDGS) ranged from 2.31 to 4.06% with a mean of 2.90%. Protein-rich feed ingredients such as, canola meal, Proplus®, casein, meat and bone meal, poultry meal contained 2.34, 1.70, 2.19, 1.17, 1.54% total RNA content, respectively while the high energy ingredients such as corn starch and dextrose contained 0.12 and 0.36% total RNA content, respectively. The *Torula yeast* RNA and the commercial nucleotide product (Nupro®) contained the highest level of total nucleotide or total RNA content among all the selected feed ingredients. Protein-rich feed ingredients contained higher total nucleotides or total RNA content as compared to the high energy ingredients.

(Key words: Nucleotide content, RNA content, feed ingredients)

APPENDIX B: THE NUTRIENT DIGESTIBILITY OF HIGH-PROETIN CORN DISTILLERS DRIED GRAINS AND THE EFFECT OF FEEDING VARIOUS LEVELS ON THE PERFORMANCE OF LAYING HENS[1]

Currently, corn fractionation technologies are being developed by some ethanol plants in an attempt to improve ethanol yield. Front-end fractionation technology involves separating the endosperm, germ, and bran fractions before fermentation. The endosperm fraction is fermented to produce ethanol and a feed coproduct, which is often called high-protein corn distillers dried grains (HP-cDDG). In general, HP-cDDG from the front-end fractionation process is higher in protein and amino acids but lower in fat and phosphorus than traditional corn distillers dried grains with solubles. In experiment 1, a total of 8 samples were analyzed for TME_N, and a total of 7 samples were analyzed for total and digestible amino acids by the precision-fed conventional rooster assay and cecectomized rooster assay, respectively. The average (and range) TME_N, protein, fat, and fiber values for 8 HP-cDDG samples were 2,851 kcal/kg (2,667 to 3,282 kcal/kg), 44.0 (42.2 to 45.9), 3.03 (1.89 to 5.40), and 7.42% (6.98 to 9.20%; as-fed basis), respectively. The average (and range) total lysine concentration and digestibility coefficient of HP-cDDG were 1.23 (1.13 to 1.38) and 76.1% (67.5 to 85.6%). Experiment 2 was conducted to evaluate the feeding value of HP-cDDG from a plant using front-end fractionation on the performance of laying hens. Five experimental diets were formulated to contain 0, 3, 6, 9, or 12% HP-cDDG. The mash diets were fed to 15 replications of 6 Hy-Line W-36 laying hens per replication from 21 to 41 wk of age. The addition of 3% HP-cDDG significantly improved egg mass as compared with eggs from hens fed the control diet. Overall, at 41 wk no difference in FE,

[1] Jung, B. and A. B. Batal. Published in *J. Appl. Poult. Res. (2009). 18:741-751.*

egg yolk color, or specific gravity was attributable to the addition of up to 12% HP-cDDG to the diets as compared with hens fed the control diet. It is important that confirmatory analysis be conducted before using a new fermentation by-product from a new plant or supplier. High-protein corn distillers dried grains is an acceptable feed ingredient when up to 12% is used in a standard laying hen diet during peak production.

(Key words: high-protein corn distillers dried grains, amino acid, digestibility, laying hen, hen-day egg production)

APPENDIX C: EVALUATION OF HIGH PROTEIN DISTILLERS DRIED GRAINS AS A FEED INGREDIENT FOR BROILER CHICKENS[2]

Two experiments (Exp) were conducted to evaluate the feeding value of high protein corn distillers dried grains (HP-cDDG) from a plant using a front-end fractionation process on broiler performance in batteries (0 to 20 d of age: Exp. 1) and floor pens (0 to 33 d of age: Exp. 2). In Exp. 1, 1-d-old male broiler chicks were placed in batteries in an environmentally controlled room. Six experimental diets were formulated to contain 0, 3, 6, 9, 12, or 16% HP-cDDG and to be isocaloric and isonitrogenous. The mash diets were fed to 8 replications of 6 chicks per replication from 0 to 20 d of age. Overall (0 to 20 d of age) the inclusion of 6% HP-cDDG or greater to the diets negatively impacted body weight (BW) gain and feed conversion ratio as compared to the control or the 3% HP-cDDG diet. The negative effect was likely due to a marginal lysine deficiency in the diets. In Exp. 2, 1-d-old male broiler chicks were housed in floor pens equipped with hanging tube feeders, a nipple watering system and pine wood shavings in an environmentally controlled room. Six experimental diets were formulated to contain 0, 3, 6, 9, 12, or 16% HP-cDDG and to be isocaloric. The crumble (0 to 15 d of age) and pellet diets (16 to 33 d of age) were fed to 8 replications per treatment containing 40 chicks per replication. There was no effect of up to 16% HP-cDDG inclusion on broiler performance during the starter period (0 to 15 d of age). However, any inclusion of HP-cDDG to the diets during the grower period (16 to 33 d of age) significantly decreased BW gain as compared to the control diet. Also, the inclusion of 9% HP-cDDG or greater to the diets negatively impacted feed conversion ratio. The reason for the depression in performance was also likely due to a lysine deficiency. In

[2]Jung, B. and A. B. Batal. Published in *Canadian J. of Ani. Sci. (2010). Vol. 90, No. 4, 505-512.*

conclusion, HP-cDDG could be an acceptable feed ingredient for broilers however special attention must be given to the amino acid levels (especially lysine) in order to prevent deficiencies.

(Key words: BW, broiler, feed conversion ratio, high protein corn distillers dried grains (HP-cDDG))

APPENDIX D: NUTRITIONAL AND FEEDING VALUE OF CRUDE GLYCERIN FOR POULTRY; 1. NUTRITIONAL VALUE OF CRUDE GLYCERIN[3]

Glycerin is the main by-product of biodiesel production and it has been speculated that it may be a good energy source in poultry diets, however there is limited knowledge on the nutritional value of crude glycerin. Thus, the objective of this study was to determine the nutritional and chemical characteristics of various glycerin samples from diverse production facilities. Seven samples of crude glycerin from a variety of biodiesel producers were analyzed for gross energy (GE) and nitrogen-corrected true metabolizable energy (TME_n). Ten crude glycerin samples were analyzed for glycerol, methanol, nutritional, and mineral composition. The GE ranged from 3,337 to 6,742 kcal/kg with a mean of 4,648 kcal/kg and TME_n ranged from 2,950 to 6,711 kcal/kg with mean of 4,206 kcal/kg. Mean concentration values for glycerol, methanol, moisture, fat, salt, and ash were 63.7, 1.33, 18.2, 8.1, 2.19, and 4.35%, respectively. Phosphorus was the highest mineral component with an average of 0.1% in the crude glycerin samples and the average calcium, potassium, copper, zinc, iron, magnesium, and manganese levels were 328, 2,090, 3.20, 5.21, 18.6, 43.6, and 1.26 ppm, respectively. The range in glycerol, methanol, moisture, fat, salt, and potassium levels were from 34.2 to 86.1%, from 0.01 to 3.10%, from 7.85 to 34.9%, from 0.01 to 30.0%, from 0.01 to 4.21%, and from 120 to 11,200 ppm, respectively. The variation observed among samples strongly indicates that confirmatory analyses should be conducted prior to the use of any crude glycerin products in poultry diets.

(Key words: Glycerin, poultry, TME_n, methanol)

[3]Jung, B. and A. B. Batal. Accepted in *J. Appl. Poult. Res. (2011)*

APPENDIX E: NUTRITIONAL AND FEEDING VALUE OF CRUDE GLYCERIN FOR POULTRY; 2. EVALUATION OF FEEDING CRUDE GLYCERIN TO BROILERS[4]

Government support and subsidies have led to the rapid growth of the biofuels industry in the U.S. Crude glycerin is the primary by-product of biodiesel production and may be used as an alternative source of energy in poultry diets. However, methanol contamination and high variations in nutrient concentration may limit its use in poultry diets. Four studies were conducted to determine the maximum crude glycerin level and the effects of varying levels of methanol concentration in crude glycerin products on broiler performance. A crude glycerin level of 5% depressed body weight (BW) gain and feed intake by 2.5% when used as supplemental energy source in a diet restricted in energy. However, in a commercial diet up to 5% crude glycerin did not negatively affect BW gain and feed intake. The supplementation of crude glycerin up to 7.5% in the energy restrictive or commercial diet did not affect feed efficiency at any period measured. There was no effect on broiler performance due to the addition of up to 10% of a crude glycerin product that contained 1.79% methanol from 0 to 14 d of age. There was also no effect on performance due to the addition of 10% crude glycerin which contained 3.10% methanol to the diet while, the addition of 10% of a different crude glycerin product with much lower methanol level (<0.01 methanol) significantly depressed BW gain but did not affect feed intake and gain:feed. However in a forth experiment, there were no negative effects on performance due to the addition of up to 10% crude glycerin to the broiler diets for both < 0.01% and 2.24% methanol containing products. In summary, broiler

[4]Jung, B. and A. B. Batal. Submitted to *J. Appl. Poult. Res.(2011)*

performance was not affected due to the addition of up to 5% glycerin regardless of the methanol concentration.

(Key words: Crude glycerin, methanol, broiler, performance)

APPENDIX F: EVALUATION OF FEEDING DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) AND CANOLA MEAL ON BROILER PERFORMANCE AND CARCASS CHARACTERISTICS[5]

An experiment was conducted to evaluate the use of distillers dried grains plus solubles (DDGS) in combination with canola meal in broiler diets. In a 3 × 2 factorial design, 1,920 one-d-old male heritage broilers were randomly assigned to the 6 dietary treatments; control, 6% DDGS, 12% DDGS, 7.5% canola meal, 6% DDGS+7.5% canola meal, and 12% DDGS+7.5% canola meal. Eight replicate pens containing 40 chicks were fed each experimental diet from 0 to 48 d of age. The diets were formulated to be isocaloric and to meet the bird's digestible amino acid requirements. Performance was evaluated at 14, 21, 35 and 48 d of age and carcass characteristics were measured at 49 d of age. Overall (0 to 48 d of age) the inclusion of up to 12% DDGS in the diet did not significantly affect BW gain or feed intake but it did negatively affect feed efficiency. The inclusion of 7.5% canola meal did not significantly affect feed efficiency but, it did significantly reduce BW gain and feed intake as compared to the controls. There was no interaction between DDGS and canola meal on BW gain, feed intake or feed efficiency. The inclusion of 7.5% canola meal in the diet of boilers negatively impacted the yield of the carcass but positively affected the yield of tenders and legs. There was no interaction of DDGS and canola meal on the carcass parameters measured except for leg and wing yield. Although not statistically significant the inclusion of DDGS or canola meal in the diet did lead to a reduction in breast meat yield; from 23.4% for the birds fed the control diet to 22.5, 22.4, and 22.5% for the birds fed 6 and 12% DDGS diets and the 7.5% canola meal diet respectively.

[5]Jung, B. and A. B. Batal. To be submitted to *J. Appl. Poult. Res*.

Careful consideration should be given when DDGS and canola meal are fed because negative effects on performance and carcass yield may be observed.

(Key words: distillers dried grain with soluble (DDGS), canola meal, broilers, carcass characteristics)

APPENDIX G: EFFECT OF EXOGENOUS ENZYME SUPPLEMENTATION ON PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILERS FED DIETS CONTAINING HIGH LEVELS OF DISTILLERS DRIED GRAINS WITH SOLUBLES [6]

An experiment was conducted to determine the effect of supplementing diets containing DDGS with exogenous enzymes on performance, gastrointestinal tract weights, and carcass characteristics of broilers. In a 3 x 2 factorial design 1,920 one-day-old male Heritage® broilers were randomly assigned to 6 dietary treatments; 0% DDGS, 0% DDGS + Hemicell®, 0% DDGS + Avizyme 1510®, 12% DDGS, 12% DDGS + Hemicell®, and 12% DDGS + Avizyme 1510®. Eight replicate pens containing 40 chicks were fed each experimental diet from 0 to 50 d of age. Performance was evaluated at 14, 21, 36 and 50 d of age, gastrointestinal tract weights were measured at 21 and 50 d of age, and carcass characteristics were measured at 51 d of age. The addition of 12% DDGS and exogenous enzymes in diets did not affect BW gain and feed intake in overall period (0 to 50 d of age). However, the birds fed the diets containing 12% DDGS had significant lower feed efficiency (gain:feed) when compared to the birds fed 0% DDGS diets while exogenous enzyme supplementation did not affect gain:feed in 0% and 12% DDGS diets from 0 to 50 d of age. From 15 to 21 d of age, the supplementation of the Hemicell® enzyme lead to a significant improvement in the gain:feed compared to broilers fed diets without any enzyme supplementation. Also, the birds fed diets with 12% DDGS showed significantly heavier relative proventriculus and small intestinal weights while, supplementation with enzymes did not affect the relative gastrointestinal tract weights at 21 d of age. At 50 d of age, the addition of 12% DDGS to the broiler diet did not affect relative intestinal tract weights but, the birds fed the diet

[6] Jung, B. and A. B. Batal. To be submitted to *J. Appl. Poult. Res*.

with enzymes supplementation had lighter relative proventriculus when compared to the birds fed diets without any enzymes. Furthermore, inclusion of 12% DDGS had significantly negative effects on the hot and chilled carcass yields as well as increase in wing yield as compared to the 0% DDGS diet. However, 12% DDGS in broiler diets did not affect the yield of the breast, tenders, or legs. The Hemicell® enzyme in 0% DDGS and 12% DDGS diets decreased tender and fat pad yield as compared to the diets without enzyme supplementation. There was no interaction between DDGS and enzymes on performance, gastrointestinal tract weights, and carcass characteristics. Therefore, careful consideration should be given when 12% DDGS is fed to broilers due to its negative effects on performance and yield of carcass observed in our present study while the addition of enzymes to the 12% DDGS diets may help reducing these negative effects.

(Key words : DDGS, broilers, enzymes, performance, carcass characteristics)