Evaluation of the Efficacy of NuPro®-Yeast Extract in Reducing Intestinal *Clostridium perfringens* Levels in Broiler Chickens

by

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Abstract

The etiological agent of necrotic enteritis (NE) is *Clostridium perfringens* (CP). Traditionally, NE is controlled with in-feed antibiotics. However, increasing consumer demand for drug-free poultry has fostered the search for non-antibiotic alternatives. Yeast extract contain nucleotides that are immunomodulatory and also essential for cellular functions. Two experiments were conducted to evaluate the efficacy of NuPro®-yeast extract in reducing intestinal CP levels in broiler chickens. In the first experiment one hundred and ninety two day-old male broiler chicks were obtained, and randomly assigned to 6 treatments in a battery cage trial. Treatment 1 (CX) consisted of chicks fed corn-soybean meal (SBM) basal diet without added bacitracin methylene disalicylate (BMD) or NuPro®. Treatment 2 (CM) consisted of chicks fed corn-SBM basal into which BMD was added at 0.055 g/kg. Treatment 3 (CN) consisted of chicks fed corn- SBM basal supplemented with NuPro® at 2% level for the first 10 days of experiment. Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks that were challenged with 3 mL of CP inoculum (~10^7 cfu/mL) on days 14, 15, and 16 of experiment, and fed diets similar to treatments 1, 2, and 3, respectively. On days 1 and 7 post-challenge, intestinal CP levels, lesion scores, and alkaline phosphatase activity (ALP) were assessed. On day 1 post-challenge, CP level in PM treatment (2.09 $\log_{10}$ cfu/g) was lower ($P < 0.05$) compared to the PX treatment (4.71 $\log_{10}$ cfu/g), but similar to PN treatment (2.98 $\log_{10}$ cfu/g). A similar trend was observed on day 7 post-challenge. NuPro® supplementation

enhanced ALP activity ($P < 0.05$) in CP-challenged chicks, and appeared to reduce intestinal lesion scores. Although dietary supplementation of NuPro® in PN treatment reduced CP levels by 1.73 $\log_{10}$ cfu/g and 0.68 $\log_{10}$ cfu/g compared to PX treatment on day 1 and day 7 post-challenge, respectively, these reductions were not statistically significant. Based on the results of this experiment, we hypothesized that extending the period of NuPro® supplementation beyond the first 10 days of life should be considered for achieving significant reduction in intestinal CP levels.

In Experiment 2, a 42-day floor pen trial was conducted. Eight hundred day old male broiler chicks were obtained from a commercial hatchery and randomly assigned to 8 treatments. Treatment 1 (CX) consisted of chicks fed corn - SBM diet without BMD or NuPro® added. Treatment 2 (CM) consisted of chicks fed corn-SBM basal into which BMD was added at 0.055 g/kg. Treatment 3 (SN) consisted of chicks fed corn-SBM basal into which NuPro® was added at 2% level for the first 10 days of the experiment. Treatment 4 (LN) consisted of chicks fed corn-SBM basal into which NuPro® was added at 2% level throughout experiment. Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks fed diets similar to those given to CX, CM, SN and LN treatments respectively, and were additionally challenged with 3.5 mL of CP inoculum (~10^8 cfu/mL) on days 14, 15, and 16 of experiment. On days 1, 7, and 21 post-challenge intestinal CP levels were assessed. Results showed that by 21 days post challenge, the NuPro®-containing diet fed throughout the experiment (PLN) significantly reduced intestinal CP levels ($P < 0.05$) by 1.50 $\log_{10}$ cfu/g compared to NuPro®-free PX treatment. Conversly, the magnitude of intestinal CP reduced in the PM (1.19 $\log_{10}$ cfu/g) and PSN (1.32 $\log_{10}$ cfu/g) treatments were not statistically significant. In

conclusion, dietary supplementation of NuPro® fed throughout the growout effectively reduced intestinal CP during broiler production cycle.

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List of Abbreviations

CP	*Clostridium perfringens*
NE	Necrotic enteritis
BMD	Bacitracin methylene disalicylate
ALP	Alkaline phosphatase
CE	Competitive exclusion
SBM	Soybean meal
FTG	Fluid thioglycolate
TSC	Tryptose sulfite cycloserine
FCE	Feed conversion efficiency
GLM	General Linear Model
CPE	*Clostridium perfringens* enterotoxin
GIT	Gastrointestinal tract
BD	Basal diet
BW	Body weight

I. INTRODUCTION

Necrotic enteritis (NE) is an important enteric disease of poultry caused by *Clostridium perfringnes* (CP) Type A. *Clostridium perfringens* is Gram positive, anaerobic, spore forming rod shaped bacteria. It is ubiquitous in nature and is normally seen at low levels in the gastrointestinal tract (GIT) of most avian species. Under favorable conditions, the bacteria proliferate to high numbers and can cause acute or chronic NE. In broilers, acute NE is characterized by depressed feed intake, anorexia, typical necrotic enteritis lesions, and mortality as high as 40%, whereas, chronic NE is charecterised by impaired feed intake and feed conversion at a rate that is difficult to notice until slaughtering and carcass processing. Consequently, high numbers of livers and carcasses are condemned due to cholangiohepatitis and low carcass weight. It is estimated that NE cost the global poultry producer around 2 billion US dollars annually.

From a food safety perspective, CP is an important food borne disease-causing bacterium in humans. According to Bos *et al.* (2005) approximately 250,000 cases of CP related food borne illnesses are reported in the United States annually. Poultry products have been identified as important sources of CP causing foodborne illnesses in humans.

Controlling intestinal CP colonization and possible NE disease in poultry is crucial because of the economic loss to poultry producers, the poor health of NE-infected birds, and the potential for a food borne illness in humans. Traditionally, NE is controlled

by antibiotics such as bacitracin, virginiamycin, and lincomycin (Feed Additive Compendium, 2010). However, the emergence of antibiotic-induced resistance in intestinal bacteria, coupled with increased governmental and consumer demand for drug-free poultry meat has led to the ban of antibiotics growth promotants in the European Union, and a gradual reduction of these antibiotics in the United States. With these restrictions search for alternative, non-antibiotic strategies for NE control is a priority for poultry scientists.

A number of non-antibiotic control strategies have been investigated in recent years. These include various prebiotics, probiotics, mannonoligosaccharides, organic acids, and enzymes. While most of these control strategies conferred some level of protection, to date, there is no single non-antibiotic alternative that provides complete protection against gastrointestinal pathogens. Hence it remains a priority for poultry scientists to identify a natural product (or a combination thereof) that can be used as a substitute to antibiotics. In the present study we evaluated the effect of a commercial yeast extract (NuPro®) on CP colonization in the intestine of broiler chickens.

Yeast extract is a natural product obtained from yeast such as *Saccharomyces cerevisiae and Kloeckera apiculata*. The effect of yeast and yeast products on improving performance parameters has been studied in various species such as ruminants, swine, and avians. In poultry, yeast supplements are effective in improving feed intake, and body weight gains (Madrigal *et al*., 1993). Dietary supplementation of yeast cultures have demonstrated some effect in modifying immune function by increasing the serum lysozyme levels, and activating natural killer cells and B lymphocytes. This immunostimulatory property of yeast could be harnessed for the control of intestinal

pathogens of importance in broiler chickens. A study conducted by Line *et al.* (1997) indicates yeast to be effective in reducing *Salmonella* and *Campylobacter* colonization in broiler chicks subjected to transport stress. The positive effect of yeast supplements on intestinal morphology by increasing the villus height and enhancing intestinal mucosal development has been demonstrated. Information is scarce or nonexistent on the effect of yeast extract supplementation on CP colonization in the intestine of broiler chickens. Thus, we conducted experiments to investigate the efficacy of NuPro®-yeast extract supplementation in reducing clinical signs associated with CP and NE disease.

II. LITERATURE REVIEW

2.1. Importance of *Clostridium perfringens* in Broiler Chickens

Clostridium perfringens (CP) is a Gram positive, anaerobic, spore forming, non motile, rod shaped bacterium (McDevitt *et al*., 2006). It is ubiquitous and occurs naturally in soil, poultry litter, feed, and decaying organic matter. The endospores formed by CP are extremely resistant to external environmental stress factors and this property enhances its ability to be wide spread in nature (Wages and Opengart, 2000). CP produces over 14 different toxins and they are classified into strains A, B, C, D, and E, based on the production of 4 of the major toxins - alpha, beta, epsilon, and iota (Petit *et al*., 1999). CP is identified as the etiologic agent in causing a variety of diseases in poultry, which includes, avian malignant disease, gizzard erosions, gangrenous dermatitis, and necrotic enteritis (Hamdy *et al*., 1983). Of these, necrotic enteritis (NE) is considered one of the severe and common forms of CP-induced diseases in poultry. The most commonly isolated CP strain in a NE outbreak is the alpha toxin-producing CP type A, although some alpha and beta toxin producing type C strains have also been reported to be associated with the disease (Truscott and Al-Sheikhly, 1977; Wages and Opengart, 2000). CP types B, D, and E are not usually related with poultry diseases.

Being a commensal, CP is found naturally in the digestive tract of most healthy animals, birds, and humans. In poultry, an average of 10^4 cfu/g of CP is normally found

in the digesta within the GIT, and this level does not appear to cause any harm to the host (Asaoka *et al* 2004). However, when the intestinal environment is altered by certain predisposing factors that favor a high frequency of adhesion of CP to the intestinal mucosa (Williams, 2005), bacteria proliferation (up to 10^7 to 10^9 cfu/g of digesta) ensues, and toxin production by CP is induced, especially in the jejunum and ileum of the small intestine (Williams, 2005).

The major factor which makes CP type A virulent to birds is still under question. It is believed that alpha toxin which is a zinc dependent phospholipase / sphingomyelinase co-enzyme is responsible for the pathogenesis of NE. This toxin has been detected in large amounts in birds suffering from NE when compared to the healthy birds (Lovland *et al* 2003). Furthermore, CP alpha toxoids have demonstrated partial or complete protection against NE in broilers (Cooper *et al*., 2009, Kulkarni *et al*., 2007). However, a recent study showed that an alpha toxin null mutant could still induce NE, challenging the theory of alpha toxin being the key virulence factor for NE. In fact, a novel toxin, NetB, has been identified as another important factor contributing to the pathogenicity of CP in birds. (Keyburn *et al*., 2008).

Necrotic enteritis is considered as one of the important enteric disease of poultry. This disease was first reported in 1961 in England, and thereafter became known in the poultry industry worldwide (Parish W. E., 1961). The estimates on the incidence of NE in the United States varies from 1- 40% (Kaldhusal and Lovland, 2000). A study conducted by Annett *et al*. (2002) estimated that up to 37% of the broiler flocks are affected by NE in the United States. The disease is manifested in two forms the clinical and subclinical. The clinical form of the disease is characterized by depressed feed intake, body weight

gain, feed efficiency, diarrhea, intestinal necrotic lesions, and high mortality rates (Al-Sheikly & Truscott, 1977; Ross Tech, 1999). The chronic or subclinical form of NE affects the welfare and productivity of birds by depressing growth and impairing feed conversion at a rate that is difficult to notice until processing. At the processing plant there would be product loss due to liver cholangiohepatitis and carcass condemnations (Stutz and Lawton, 1984., Lovland and Kaldhusdal, 1999). Cholangiohepatitis and resulting carcass condemnations contribute up to one-fifth of the loss attributed to NE in the poultry industry (Lovland and Kaldhusdal, 2001). It has been estimated that subclinical NE costs a producer about 5 cents per bird (Van der Sluis, 2000), thus reinforcing the importance of NE as a disease of poultry. The global cost of NE to the poultry industry is estimated to be 2 billion US dollars annually.

2.2. Role of *Clostridium perfringens* in Food borne Infections

From a food safety perspective, CP is an important foodborne disease-causing bacterium in humans. Approximately 250,000 cases of CP-related food borne illness are reported in the United States annually (Bos *et al*., 2005). CP type A carrying a chromosomal *cpe* gene is the etiologic agent for CP-induced gastroenteritis (a mild form of diarrhea) that is commonly associated with food poisoning in humans, while some CP type C strains have been implicated in causing NE in humans (Brynestad and Granum, 2002). It is generally accepted that cross-contamination of poultry meat by enteric pathogens of poultry such as CP, occur primarily during slaughter in the processing facility through leakage of crop contents or feces onto broiler carcass, and accidental rupture of intestinal tract by the equipment, thereby resulting in a health risk to

individuals that consume the contaminated meat products (Hargis *et al*., 1995; Zhu *et al*., 1999). In addition, CP spores are known to have an exceptional resistance to heat, which favors their growth under conditions of inadequate cooking and improper storage (Sarker *et al*., 1999). CP can grow at temperatures between 15° and 50°C with optimum growth at 45°C for most strains. They have also shown a generation time of less than 20 minutes (Labbe, 2000). These contributing factors favor the proliferation of CP in meat and meat products, thereby increasing the propensity of undercooked CP-contaminated meat to cause foodborne illness when consumed.

Poultry products have been identified as important sources of CP causing foodborne illnesses in humans (Hook *et al*., 1996). CP type A food poisoning develops when an individual consumes food containing large quantities of vegetative cells. Ingestion of food containing at least 10^6 cfu/g of vegetative cells of CP is necessary for the production of enterotoxin (CPE) in the small intestine (Brynested and Granum, 2002). When CP sporulates, they produce an enterotoxin (CPE) in the small intestine. The CPE employs multiple steps to produce resulting symptoms of the disease. Many mammalian cell types can rapidly bind to CPE (McClane, 1994). Specific binding of CPE to its receptors is the first step in CPE-induced cytotoxicity (McDonel and McClane, 1979). The enterotoxin first binds to the receptors (which are usually members of Claudine family) to form a small complex. The small complex oligomerizes into CPE hexamer 1 (Robertson *et al*., 2007), which inserts into the plasma membrane of the target cell, creating a pore that allows the influx of calcium into the cell (McClane, 1994). This induces cell death by either apoptosis or oncosis (Chakrabarthi, 2003). Symptoms such as acute abdominal pains, nausea, and diarrhea are noticed 8 to 12 hours after the ingestion of contaminated

food. The disease is usually self limiting and lasts for approximately 24 hours. Complications or death is very rare, and may occur in elderly or immunocompromised individuals (Andersson *et al*., 1995). CP type C-induced NE is mainly due to beta toxins, but gamma and iota toxins are also associated with this bacterium. Symptoms are usually noticed 5 to 6 hours after ingestion of CP. There is an acute onset of abdominal pains, bloody diarrhea, followed by NE, and is often fatal with a mortality rate of 15 to 25% (Granum, 1990). This is a very rare type of illness in industrialized world but commonly reported in some developing countries (e.g. Papua New Guinea) with severely malnourished population. (Lawrence and Walker, 1976). The disease often occurs in individuals with low levels of proteolytic enzymes due to lower protein intake and also consumption of sweet potatoes which contains trypsin inhibitors. The β toxins are not degraded due to decreased levels of proteolytic enzymes thereby resulting in the disease (Granum, 1990).

2.3. Epidemiology and Predisposing Factors of Necrotic Enteritis in Avian Species

NE affects a wide species of birds. It has been reported in broilers (Cowen *et al*., 1987), layers (Broussard *et al*., 1986), turkeys (Droual *et al*., 1995), and quails (Berkhoff, 1985). NE outbreaks in broiler usually occur when the flocks is around 2-6 weeks of age (Long, 1973). The disease has also been reported in 3-6 months old commercial layers (Porter, 1998), and 7-12 weeks old turkeys (Songer, 1996). In poultry, NE is mainly propagated through horizontal transmission. For example, day-old chicks pick up CP from the hatchery environment. The ingested CP colonizes and slowly establishes a subclinical infection in which the bacteria and its spores are continuously shed in the

feces, thus contaminating the environment. During this process, additional chicks in the flock are exposed to CP, leading to wide spread infection. In addition, CP can be present in feed and litter. Existence of a conducive intestinal environment will favor the proliferation of CP, resulting in NE outbreaks (Craven *et al*., 2001). Vertical transmission of CP from parents to progeny has also been suggested (Williams, 2002). Although the factors contributing to the onset of CP-induced NE is still under investigation, NE outbreak is always characterized by an increase in intestinal CP and alpha toxin levels. Predisposing factors that make the GIT environment favorable for the proliferation of pathogen are briefly described below.

2.3.1. Dietary Factors

A strong correlation between the type and amount of dietary ingredients and the incidence of NE has been demonstrated by various researchers. Cereals such as wheat, barley, and rye which contain indigestible water soluble non-starch polysaccharides such as β-glucans and arabinoxylans, favor the development of NE (Hofshagen and Kaldhusdal, 1992; Annett *et al*., 2002). Kaldhusdal and Skjerve (1996) observed that incidence of CP is associated with the type of cereal (maize, wheat, or barley) in poultry diets when they conducted a study on two major epidemics of NE in Norway. They found that maize was beneficial whereas wheat and barley were risk factors. High levels of animal protein is also reported to increase the incidence of NE. Dahiya *et al*., (2005) reported that high glycine levels in animal protein favors the growth of CP. Also diets containing high levels of fish meal (Drew *et al*., 2004) and potato protein (Wiclkie *et al*., 2005) were favorable for proliferation of CP. Diets rich in proteins are not digested and

absorbed well by the upper GIT. Hence, when these compounds and their metabolites reach the lower GIT, they act as substrates for the proliferation of various gut microflora. Also, nitrogenous degraded products increases the pH, thereby counteracting the acidity created by acetic and lactic acid producing bacteria, hence favoring the proliferation of CP (Juskiewicz *et al*., 2004). Increased viscosity of the digesta due to inclusion of water soluble, indigestible non-starch polysaccharides in the diet also favors the proliferation of CP (Kocher, 2003).

2.3.2. Co-infection with *Coccidia*

Intestinal coccidiosis is considered a major factor predisposing commercial broiler chickens to NE (Shane *et al*, 1985). Some of the early NE outbreaks were associated with coccidia co-infection. *Eimeria brunetti* and *Eimeria maxima* are two coccidia species reported to be commonly associated with CP infection (Nairn and Bamford, 1967; Hemboldt and Bryant, 1971). The intestinal mucosal damage caused by the sporozoites and merozoites together with the decreased intestinal pH provides an environment for the proliferation and establishment of CP infection (Johansson and Sarles, 1948; Baba *et al*., 1997). It has also been demonstrated that CP adheres at a faster rate to the cecal mucosa that is damaged by *Eimeria* spp. than to a mucosa that is not damaged (Baba *et al.*, 1992). There is a 25% increase in mortality rate when NE occurs with *Eimeria* co-infection (Drew *et al.*, 2004).

2.3.3. Miscellaneous Factors

There are a number of other stress factors that have the potential to alter the intestinal environment, in turn allowing CP to colonize. Sudden alterations in feeding regimes, shift from starter to grower diets, physical damage of the gastrointestinal lining by rough litter, and a variety of environmental and management factors can induce stress and thereby favor NE outbreak (Drew *et al*., 2004; McDevitt *et al*., 2006; Pedersen *et al*., 2008). Irrespective of the predisposing factors, the resulting intestinal damage initiates a cascade of physiological changes that provide an intestinal environment that is conducive for the proliferation of CP. Regardless of the variability in susceptibility to the disease, it has been reported that up to 75-95 % of birds are colonized by CP, thus indicating the need for continuous control of the disease (McDevitt *et al*., 2006).

2.4. Pathogenesis of Necrotic Enteritis

The disease is manifested in two forms - the clinical (or acute) form and the subclinical (or chronic form). Clinical NE is characterized by depression, ruffled feathers, anorexia and diarrhea (Al-Sheikly and Truscott, 1977; Ross Tech, 1999). Typical NE lesions are more pronounced in the jejunum and ileum, when compared to the duodenum and ceca (Long *et al*., 1974; McDevitt *et al*., 2006). Gross lesions are also noticed in other organs such as liver and kidney. The intestinal wall becomes thin and gets distended with gas (Broussard *et al*., 1986). Microscopic examination of a necrotic intestinal mucosa has revealed villi necrosis, cellular degeneration at the level of submucosa or muscularis mucosa, and coagulation of necrotized villus tip, all constituting a grey brown to yellow green diphteric membrane covering the intestinal

mucosa (Gazdinski and Julian, 1992). The important economic factor during clinical NE is that it causes high mortality rate of about 1% per day (Helmboldt and Bryant, 1999) or 40% over the 7 to 8 week broiler production cycle (Ross Tech, 1999).

The subclinical form of NE is characterized by depressed performance and focal NE lesions in the intestine (Lovland and Khaldhusdal, 2001). In addition, CP gains access into the portal system and cause cholangiohepatitis in the livers – a condition characterized by a pale and enlarged livers. In broilers, microscopic examination of the livers reveals bile duct hyperplasia, fibrinoid necrosis and cholangitis (Randall *et al*., 1983; Lovland and Khaldhusdal, 1999). Overall, subclinical NE affects the welfare and productivity of birds by depressing growth and impairing feed conversion at a rate that is difficult to notice during rearing (production) but is detected when the birds are slaughtered and processed. During processing of broilers, low carcass weights are observed, resulting in condemnation of a high number of carcasses along with cholangiohepatitic livers (Stutz and Lawton, 1984., Lovland and Kaldhusdal, 1999).

Variability in the intestinal microenvironment of the chicken and the severity of pre-existing conditions presents difficulty in experimentally reproducing NE infection in chicken. Regardless, the following approaches are confirmed to consistently produce clinical or sub-clinical NE; i) Spontaneous challenge model in which broth culture of CP is mixed with feed and fed to birds for one to three consecutive days (Kaldhusdal *et al*., 1999; Long and Truscott, 1976; Al Skeikhly and Truscott, 1977; Cowen *et al*., 1987; Branton *et al*., 1997), ii) The *Eimeria*-CP challenge model in which sporulated *Eimeria* oocyst are orally inoculated when chicks are 7 days of age, and followed by CP challenge on days 11, 12 and 13 (Shane *et al*., 1985; Baba *et al*., 1992; Baba *et al*., 1997; Jackson *et*

al., 2003; Williams *et al*., 2003), iii) Infected litter model in which birds are raised on built up litter from a broiler flock that previously had NE (Hamdy *et al*., 1983), iv) Altering dietary crude protein and protein source to predispose chicken to CP (Drew *et al*., 2004), and v) Oral gavaging of CP cultures (Siragusa *et al*., 2008). Any one of the above approaches could be employed to produce a successful experimental infection.

2.5. Control of Necrotic Enteritis in Broiler Chickens

NE is considered a complex and multifactorial disease whose pathogenesis is not well understood. Traditionally, anticoccidials (monensin and salinomycin) and antibiotic growth promoters such as bacitracin (Bernnan *et al*., 2003), virginiamycin (George *et al*., 1982), and lincomycin (Hamdy *et al*., 1983) are used to control NE in poultry (United States Feed Additive Compendium, 2010). During the last decade, there has been an increase concern about the impact of in-feed antibiotics on human health. The use of antibiotics in livestock feed has been implicated as a contributing factor to the development of resistant bacterial strains that sometimes find their way into the food chain and cause human illnesses that do not generally respond to antibiotic therapy (Diarra *et al*., 2007). This has contributed to the ban of antimicrobial feed additives in the European Union, and an increasing governmental and consumer pressure to halt antibiotic addition into feed of poultry and other food animals here in the USA. Consequently, several alternative non-antibiotic strategies are being investigated for NE control. These non-antibiotic control strategies are discussed in the next sections.

2.5.1. Competitive Exclusion (CE)

A CE product is a suspension of GIT microflora that is obtained from a healthy adult chicken (Craven *et al*., 1999). The CE culture is fed to newly hatched chicks on the hypothesis that a favorable adult microflora will be quickly established and in turn delay or prevent the colonization of pathogens such as *Salmonella* spp. and *Clostridium* spp. Use of various CE cultures have decreased the incidence of NE, reduced lesion scores and mortality, and have also enhanced performance parameters in various broiler chicken experiments (Hofacre *et al*., 1998, Craven *et al*., 1999).

2.5.2. Probiotics

Probiotics are live cultures of bacteria (such as *Bacillus*, *Lactobacillus*, *Enterococcus*) and yeast (*Saccharomyces)* which improves intestinal microbial balance and reduces the levels of enteric pathogens in the GIT. The mechanism of action is brought about by competition for the colonization sites in the gut, production of volatile fatty acids, and stimulation of the host immune system (Doyle and Erickson 2006). Although information is scanty on the effect of probiotics on intestinal CP colonization in chickens, a number of reports have documented the efficacy of probiotics in reducing intestinal levels of *Salmonella* and *Campylobacter* (Pascual *et al*., 1999). In the studies conducted by Hofacre *et al*., (1998, 2003), probiotics demonstrated some effect in reducing NE lesions and decreasing mortality rates from 60% to 30% in day-old chicks. The level of protection offered was not considered to be as effective as CE cultures.

2.5.3. Prebiotics

Prebiotics are non digestible complex carbohydrates such as fructo-oligosaccharides and mannan oligosachharides that are added to the feed (Doyle and Erickson, 2006). They act as substrates for the growth of beneficial bacteria in the gut (Gibson and Roberfroid, 1995). Most of the research on the effect of prebiotics on CP is done in *in-vitro* systems, and did not produce consistent results (Bello *et al*., 2001; McBain and MacFarlane, 2001). However, a study conducted by Branton *et al*., (1997) demonstrated that complex carbohydrates are effective in reducing NE lesions in broilers.

2.5.4. Lactose

Lactose is a disaccharide that acts as a substrate for the proliferation of beneficial bacteria. It also reduces the pH in the intestine, making the intestinal environment unfavorable for the proliferation of CP (Nisbet *et al*., 1993). Takeda, *et al*., (1995) demonstrated that 2-10% w/v lactose solution was effective in reducing CP level in the ceca of broiler chicks. However, Stringfellow *et al*., (2009) did not find any significant difference in bacterial colonization but found a decrease in NE lesions when they fed a combination of bismuth citrate (100 ppm) and dietary lactose in broilers.

2.5.5. Enzymes

Enzymes such as xylanases and β-glucanases are now widely used in broiler production. The non-starch polysaccharides in the diet are broken down by these enzymes resulting in various end products that enhance gut microbial profile and

intestinal integrity (Chesson**,** 2001). Furthermore, these enzymes reduce the viscosity of the digesta, thus preventing the adhesion of CP to the intestinal wall (Bedford, 2000).

2.5.6. Organic acids

Organic acids such as propionic acids can be added as feed or water supplements, or are incorporated into the litter (Garrido *et al*., 2004). Basically these acids lower the pH of the gut thereby favoring the growth of beneficial bacteria and inhibiting the growth of CP. A study conducted by Gornowicz (2004) proved organic acids to be effective in reducing CP levels in litter when supplemented in poultry diets.

2.5.7. Alteration in dietary regimes

A strong correlation between dietary factors and NE has been reported by various authors. Feeding corn is found to be beneficial whereas wheat, rye, oats and barley are considered risk factors (Engberg *et al*., 2004; Branton *et al*., 1987; Riddell and Kong, 1992). High protein content especially bone meal and animal protein such as fish meal increases the incidence of NE (Drew *et al*., 2004). Dietary fat is also considered a factor whose inclusion levels should be restricted. Animal fat such as lard and tallow was found to increase CP levels when compared to soy oil (Knarreborg *et al*., 2002).

Form of feed is also reported to influence the incidence of NE. Branton *et al*. (1987) suggested that finely ground feed favors NE compared to coarsely ground feed. However, Engberg *et al*. (2002) did not find any relationship between finely ground and coarsely ground feed, but did observe that pelleted feed decreased CP levels when compared to mash feed.

2.5.8. Bacteriophage

Recently, the effect of bacteriophage against various pathogens has gained interest. A study by Zimmer *et al*. (2002) confirmed bacteriophage to be an effective anti-clostridial agent *in vitro*. Information is non-existent regarding the effect of bacteriophage on CP colonization in the chicken intestine.

2.5.9. Vaccination

Vaccination has been identified as the most cost-effective approach for prophylactic control of infectious diseases in livestock and poultry (Babiuk *et al*., 2003). Lovland *et al* (2004) tested the effect of a vaccine based on CP type A and C toxoid on broiler chickens and found some level of protection against subclinical NE. Netvax® (CP type A toxoid vaccine) is another vaccine that is intramuscularly or subcutaneously administered to breeder hens for protection against NE. According to the manufacturer (Netvax®, Schering-Plough Animal Health Corporation, Omaha, Nebraska), the vaccine is administered to breeder hens at 10 to 15 weeks of age, followed by revaccination at 17 to 20 weeks of age for the induction of high antibody titers (against the alpha toxin of CP) that are transferred to progeny chicks via the egg yolk. Vaccination of chicks against CP is considered a promising strategy for the control of NE in poultry. However, to date, there is no commercially available anti-clostridia vaccine that imparts full protection against NE in poultry.

In summary, researchers have worked on various alternative strategies such as probiotics/direct fed microbial products (Elwinger *et al*., 1992), prebiotics (Branton *et al*., 1997), Beta mannase feed enzyme (Jackson *et al*., 2003), alterations in diet formulation

and ingredient selection (Engberg *et al*., 2004), and vaccines (Lovland *et al*., 2004). While these alternative therapies are somewhat beneficial, so far, none of them have been able to furnish complete protection against NE in poultry. Hence, it remains a priority for poultry scientists to identify novel effective products or a combination thereof that can be used as a substitute to antibiotics.

2.6. Effect of Yeast Extract on Enteric Pathogens in Poultry

Yeast extract is a natural product obtained from yeast such as *Saccharomyces cerevisiae and Kloeckera apiculata*. It is commonly used in human diets as food additives or flavoring agents. The beneficial effect of supplementing yeast in animal feed has been identified since 1925 (Eckles and Williams., 1925). The effect of yeast and yeast products on improving performance parameters has been studied in various species such as ruminants (Jouany, 2001), swine (Shin *et al*., 2005), and avians (Douglas *et al*., 2003). In poultry, yeast supplements are effective in improving feed intake, and body weight gains (Madrigal *et al*., 1993). Dietary supplementation with yeast cultures have demonstrated an effect in modifying immune function in some animal species by increasing the serum lysozyme levels, and activating natural killer cells and B lymphocytes. (Jensen *et al*., 2008; Goa *et al*., 2008). This immunostimulatory property of yeast could be harnessed for the control of intestinal pathogens in broiler chickens. The immunostimulatory effect of yeast extract is suggested to be brought about by its nucleotide content (Savange and Zakerwska, 1996). A study conducted by Line *et al*. (1997) indicates yeast to be effective in reducing *Salmonella* and *Campylobacter* colonization in broiler chicks subjected to transport stress. In another study, yeat extract supplemented at the rate of 1.0 g/kg of feed

in turkey poults had significantly enhanced lamina propria thickness, crypt depth, and mucin-producing goblet cells in the ileum, and to some degree in the jejunum and duodenum, compared to the control group (Santos *et al*., 2007). Thickness of the lamina propria is used as an indicator of gut health as it consists of dendritic cells that protects the gut against infection by stimulating the adaptive immune response, increasing gut motility, and modifying mucin and IgA production (Macpherson and Harris, 2004). The stem cells in the crypts of the villi are the precursors for several specialized cells, including absorptive enterocyte, goblet cells, enteroendocrine cells and paneth cells. Goblet cells secrete glycoprotein compound known as mucins (Forstner, 1978), which layers the luminal surface to protect the gut from invasion of enteric bacteria that damages the mucosa. (Specian and Oliver, 1991). Paneth cells produces antibacterial pepetides, digestive enzymes and growth factors (Ouellette and Selsted, 1996). Since CP is an enteric pathogen that cause extensive damage to the intestinal epithelium in broiler chicks, dietary supplementation of yeast extract into broiler chick diets as a non-antibiotic feed supplement could reduce the lesions associated with CP colonization in the intestine of broiler chicks.

2.7. Research Objectives

The goal of this study was to evaluate the efficacy of NuPro®-yeast extract supplementation in reducing clinical signs associated with CP and NE disease.

The first experiment was a 25 day trial, with three diets formulated to compare the effect of NuPro® yeast extract supplementation in reducing CP levels in the intestine of broiler chickens. A control diet with corn SBM basal only, a corn SBM basal

supplemented with BMD, and a corn SBM basal supplemented with NuPro®. The effect of NuPro® versus BMD on reducing intestinal CP levels was evaluated by experimentally inducing CP infection in the intestine of chicken and sampling on day 1 and day 7 post challenge. Intestinal lesion scores and jejunal alkaline phosphates levels were assessed to estimate the degree of clinical signs associated with CP. Bird live performance parameters were evaluated to compare the effect of NuPro® and BMD on body weight, and feed conversion ratios on days 7, 14,and 21 of the experiment.

The second experiment was a 42 day floor pen trial where chicks were raised on litter to closely simulate poultry production systems. The effect of NuPro® versus BMD on intestinal CP levels was evaluated by sampling on day 1, day 7 and day 21 post challenge. Bird live performance parameters were evaluated to compare the effect of NuPro® and BMD on body weight, percent uniformity and feed conversion ratios on days 21 and 42 of the experiment.

2.8. References

Al-Sheikhly, F., and R. B.Truscott. 1977. The pathology of necrotic enteritis of chickens following infusion of crude toxins of *Clostridium perfringens* into the deodenum. Avian Dis. 21:241–255.

Andersson, A., U. Ronner, and P. E. Granum. 1995. What problems does the food industry have with the spore-forming pathogens *Bacillus cereus* and *Clostridium perfringens*? Int. J. Food Microbiol. 28:145–156.

Annett, C. B., J. R. Viste, M. Chirino Trejo, H. L. Classen, D. M. Midddleton, and E. Simko. 2002. Necrotic enteritis: effects of barley, wheat and corn diets on proliferation of *Clostridium perfringens* type A. Avian Pathol. 31: 599–602.

Asaoka, Y., T. Yannai, H. Hirayama, Y. Une, E. Saito, H.Sakai, M.Goryo, H. Fukushi, and T. Masegi.2004. Fatal necrotic enteritis associated with *Clostridium perfringens* in wild crows (*Corvus macrorhynchos*). Avian Pathol. 33:19–24.

Baba, E., T. Ikemoto, T. Fukata, K. Sasai, A. Arakawa, and L. R. McDougald. 1997. Clostridial population and the intestinal lesions in chickens infected with *Clostridium perfringens* and *Eimeria necatrix*. Vet. Microbiol. 54:301–308.

Baba, E., H. Wakeshima, K. Fukui, T. Fukata, and A. Arakawa.1992. Adhesion of bacteria tothe cecal mucosal surface of conventional and germ-free chickens infected with *Eimeria tenella*. Am. J. Vet. Res. 53:194–197.

Babiuk, L.A., S. Gomis, and R. Hecker. 2003. Molecular approaches to disease control. Poult. Sci. 82:870-875.

Bedford, M. 2000 Removal of antibiotic growth promotors from poultry diets: implications and strategies to minimise subsequent problems. World's Poult. Sci. J. 56:347–365.

Bello, F. D., Walter, J., Hertel, C, and W. P. Hammes 2001. In vitro study of prebiotic properties of levan-type exopolysaccharides from *Lactobacilli* and non-digestible carbohydrates using denaturing gradient gel electrophoresis. Syst. Appl. Microbiol. 24:232– 237.

Berkhoff, H. A. 1985. *Clostridium colinum* sp. now, the causative agent of ulcerative enteritis (quail disease) in quail, chickens, and pheasants. Int. J. Syst. Bacteriol. 35:155–159.

Branton, S. L., Reece, F. N, and W. M. Jr. Hagler 1987. Influence of a wheat diet on mortality of broiler chickens associated with necrotic enteritis. Poult. Sci. 66:1326–1230.

Branton, S. L., B.D.Lott., L.W.Deaton., W.R.Maslin., F.W.Austin, L.M.Pote., R.W.Keirs., M. A. Latour., and E. J. Day. 1997. The effect of added complex carbohydrates or added dietary fibers on necrotic enteritis lesions in broiler chicken. Poult. Sci. 76:24–28.

Brennan, J., J. Skinner, D. A. Barnum, and J. Wilson. 2003. The Efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poult. Sci. 82:360–363.

Broussard, C.T., C. L. Hofacre, R. K. Page, and O. J. Fletcher. 1986. Necrotic enteritis in cage-reared commercial layer pullets. Avian Dis. 30:617–619.

Brynestad, S., and P. E. Granum. 2002. *Clostridium perfringens* and food borne infections. Int. J. Food Microbiol.74:195–202.

Bos, J., L. Smithee, B. McClane, R. F. Distefano, F. Uzal, J. G. Songer, S. Mallonee. 2005. Fatal necrotizing colitis following a foodborne outbreak of enterotoxigenic *Clostridium perfringens* type A infection. Clin. Infect. Dis. 40:e78–83.

Chakrabarti, G., X. Zhou, and B. A. McClane**.** 2003. Death pathways activated in CaCo-2 cells by *Clostridium perfringens* enterotoxin. Infect. Immun. 71**:**4260–4270.

Chesson, A. 2001. Non-starch degrading enzymes in poultry diets: influence of ingredients on the selection of activities. World's Poult. Sci. J. 57:251-263.

Cooper, K. K., H. T. Trinh, and J. G. Songer. 2009. Immunization with recombinant alpha toxin partially protects broiler chicks against experimental challenge with *Clostridium perfringens.* Vet Microbiol 133:92–97.

Cowen, B. S., L. D. Schwartz, R. A. Wilson, and S. I. Ambrus. 1987. Experimentally induced necrotic enteritis in chickens. Avian Dis. 31:904–906.

Craven, S. E., N. J. Stern, N. A. Cox, J. S. Bailey, and M. Berrang. 1999. Cecal carriage of *Clostridium perfringens* in broiler chickens given mucosal starter culture. Avian Dis. 43:484–490.

Craven, S. E., N. J. Stern, S. J. Bailey, and N. A. Cox. 2001. Incidence of *Clostridium perfringens* in broiler chickens and their environment during production and processing. Avian Dis.45:887–896.

Dahiya, J. P., D. Hoehler, C. Wilkie, A.G. Van Kessel, and M.D. Drew. 2005. Dietary glycine concentration affects intestinal *Clostridium perfringens* and *Lactobacilli* populations in broiler chickens. Poult. Sci. 84:1875–1885.

Diarra, M. S., F. G. Silversides, F. Diarrasouba, J. Pritchard, L. Masson, R. Brousseau, C. Bonnet, P. Delaquis, S. Bach, B. J. Skura, and E. Topp. 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and *Enterococcus* counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. Appl. Environ. Microbiol. 73:6566–6576.

Douglas, M. W., M. Persia, and C. M. Parsons 2003. Impact of galactose, lactose, and Grobiotic-B70 on growth performance and energy utilization when fed to broiler chicks. Poult. Sci. 82:1596–1601.

Doyle, M .P, and M. C. Erickson. 2006. Reducing the carriage of foodborne pathogens in livestock and poultry. Poult. Sci. 85: 960–973.

Drew M. D., N. A. Syed, B. G. Goldade, B. Laarveld, and A. G. Van Kessel 2004. Effects of dietary protein source and level on intestinal populations of *Clostridium perfringens* in broiler chickens. Poult. Sci. 83:414–420.

Droual, R., T. B. Farver, and A. A. Bickford. 1995. Relationship of sex, age, and concurrent intestinal disease to necrotic enteritis in turkeys. Avian Dis. 39:599–605.

Eckles, C. H., and V. M. Williams. 1925. Yeast as a supplementary feed for lactating cows. J. Dairy Sci. 8:89–93.

Elwinger, K., C. Schneitz, F. Berndtson, O. Fossum, B. Teglof and B. Bagstrom. 1992. Factors affecting the incidence of necrotic enteritis, cecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. Acta. Vet. Scand. 33:369–378.

Engberg, R. M, M. S. Hedemann, T. D. Leser, and B. B. Jensen. 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci. 79:1311–1319.

Engberg, R. M., M. S. Hedemann, and B. B. Jensen. 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Br. Poult. Sci. 44:569–579.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925–938.

Forstner, J. T. 1978. Intestinalmucins in health and disease. Digestion 17:234–263.

Fukata, T., Y. Hadate, E. Baba, and A. Arakawa. 1991. Influence of bacteria on *Clostridium perfringens* infection in young chickens. Avian Dis. 35:224–227.

Gao, J., H. J. Zhang, S. H. Yu, S. G. Wu, I. Yoon, J. Quigley, Y. P. Gao, and G. H. Qi. 2008. Effects of Yeast Culture in Broiler Diets on Performance and Immunomodulatory Functions. Poult. Sci. 87:1377–1384.

Garrido, M. N., M. Skjervheim, H. Oppegaard, and H. Sorum. 2004. Acidified litter benefits the intestinal flora balance of broiler chickens. Appl. Environ. Microbiol. 70:5208–5213.

Gadzinski, P., and R. J. Julian. 1992. Necrotic enteritis in turkeys. Avian Dis. 36:792–798.

George, B. A., C. L Quarks, and D. J. Fagerberg. 1982. Virginiamycin effects on controlling necrotic enteritis infection in chicken. Poult. Sci. 61:447–450.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125:1401.

Gornowicz, E. 2004. Microbiological evaluation of poultry house environments as influenced by acidifying preparations added to compound feed. Med. Vet. 60: 755–758.

Granum, P. E., 1990. *Clostridium perfringens* toxins involved in food poisoning. Int. J. Food Microbiol. 10:101–112.

Hamdy, A. H., R. W. Thomas, D. D. Kratzer, and R. B. Davis.1983. Lincomycin dose response for treatment of necrotic enteritis in broilers. Poult. Sci. 62:585–588.

Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. Poult. Sci. 74:1548–1552.

Helmboldt, C. F., and E. S. Bryant. 1971. The pathology of necrotic enteritis in domestic fowl. Avian Dis. 15:775–780.

Hofacre, C. L., R. Froyman, B. Gautrias, B. George, M. A. Goodwin, and J. Brown.1998. Use of Aviguard and other intestinal bioproducts in experimental *Clostridium perfringens* associated necrotizing enteritis in broiler chickens. Avian Dis. 42:579–584.

Hofacre, C. L., T. Beacorn, S. Collett, and G. Mathis. 2003. Using competitive exclusion, mannan-oligosaccharide and other intestinal products to control necrotic enteritis. J. Appl. Poult. Res. 12:60–64.

Hofshagen, M., and M. Kaldhusdal, M. 1992. Barley inclusion and avoparcin inclusion in broiler diets. Effect on small intestinal bacterial flora and performance Poult. Sci. 71:959–969.

Hook, D., B. Jalaludin, and G. Fitzsimmons. 1996. *Clostridium perfringens* food borne outbreak : an epidemiological investigation. Aust. Nz. J. Publ. Heal. 20:119–122.

Jackson, M. E., D. M. Anderson, H. Y. Hsiao, G. F. Mathis, and D. W. Fodge. 2003. Beneficial effect of beta mannanase feed enzyme on performance of chicks challenged with *Eimeria* sp. and *Clostridium perfringens*. Avian Dis. 47:759–763.

Jensen, G. S., K. M. Patterson, and I. Yoon. 2008. Yeast culture has anti-inflammatory effects and specifically activates NK cells. Comp. Immunol. Microbiol. Infect. Dis. 31:487–500.

Johansson, K. R., and W. B. Sarles. 1948. Bacterial population changes in the ceca of young chickens infected with *Eimeria tenella*. J. Bacteriol. 56:635–647.

Jouany, J. P. 2001. A new look to at yeast culture as probiotics for ruminants. Feed Mix. 9:17–19.

Juskiewicz, J., Z. Zdunczyck, and J. Jankowski. 2004. Selected parameters of gastrointestinal tract metabolism of turkeys fed diets with flavomycin and different inulin content. World's Poult. Sci. J.60:177–185.

Kaldhusdal M., M. Hofshagen, A. Lovland, H. Langstrand, and K. Redhead. 1999. Necrotic enteritis challenge models with broiler chickens raised on litter: evaluation of preconditions, *Clostridium perfringens* strains and outcome variables. FEMS Immunol. Med. Microbiol. 24:337–343.

Kaldhusdal, M., and E. Skjerve. 1996. Association between cereal content in the diet and incidence of necrotic enteritis in broiler chickens in Norway. Prev. Vet. Med. 28:1–16.

Kaldhusdal, M., and Lovland. 2000. The economic importance of *Clostridium perfringens* is greater than anticipated. World's Poult. Sci. 16:50–51.

Keyburn, A. L., J. D. Boyce, P. Vaz, T. L. Bannam, M. E. Ford, D. Parker, A. Di Rubbo , J. I. Rood, and R. J. Moore. 2008. NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens*. Public library of science Pathog. 4(2):e26.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microb. 68:5918–5924.

Kocher, A., M. Choct, G. Ross, J. Broz, and T. K. Chung. 2003. Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. J. Appl. Poult. Res. 12:275–283.

Kulkarni, R. R., V. R. Parreira, S. Sharif, J. F. Prescott. 2007. Immunization of broiler chickens against *Clostridium perfringens*-induced necrotic enteritis. Clin. Vaccine. Immunol. 14:1070–1077.

Labbe´, R.G., 2000. *Clostridium perfringens*. In: Lund, B., Baird-Parker, T., Gould, G. (Eds.), The Microbiological Safety and Quality of Food. Aspen Publishers, MD, USA, 1110–1135.

Lawrence, G., and P. D. Walker. 1976. Pathogenesis of enteritis necroticans in Papua New Guinea. Lancet 1 (7951), 125–126.

Line, E. J., J. S. Bailey, N. A. Cox, and J. S. Norman. 1997. Yeast treatment to reduce *Salmonella* and *Campylobacter* populations associated with broiler chickens subjected to transport stress. Poult. Sci. 76:1227–1231.

Line, E. J., J. S. Bailey, N. A. Cox, J. N. Stern, and T. Tompkins. 1998. Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. Poult. Sci. 77:405–410.

Long, J. R. 1973. Necrotic enteritis in broiler chickens. I. A review of the literature and the prevalence of the disease in Ontario. Can. J. Comp. Med. 37:302–308.

Long, J. R., J. R. Petit, D. A. Barnum. 1974. Necrotic enteritis in broiler chicken ii. Pathology and proposed pathogenesis. Can. J. Comp. Med. 38:467–474.

Long J. R., R. B. Truscott. 1976. Necrotic enteritis in broiler chickens. III. Reproduction of the disease. Can J Comp. Med. 1976:40:53–59.

Lovland, A., and M. Kaldhusdal. 1999. Liver lesions seen at slaughter as an indicator of necrotic enteritis in broiler flocks. FEMS Immunol. Med. Micbiol. 24:345–351.

Lovland, A., and M Kaldhusdal. 2001. Severely impaired production performance in broiler flocks with high incidence of *Clostridium perfringens*-associated hepatitis. Avian Pathol. 30:73-81.

Lovland, A., M. Kaldhusdal, and L. J. Reitan. 2003. Diagnosing *Clostridium perfringens* associated necrotic enteritis in broiler flocks by an immunoglobulin G antialpha-toxin enzyme-linked immunosorbent assay. Avian Pathol. 32:527–534.

Lovland, A., M. Kaldhusdal, K. Redhead, E. Skjerve, E and A. Lillehaug. 2004. Maternal vaccination against subclinical necrotic enteritis in broilers. Avian Pathol. 33: 81–90.

Macpherson, A. J., and N. L. Harris. 2004. Interactions between commensal intestinal bacteria and the immune system. Nat. Rev. Immunol. 4:478–485.

Madrigal, S. A., S. E. Watkins, J. T. Skinner, M. H. Adams, A. L. Waldroup, and P. W. Waldroup, 1993. Effect of an active yeast culture on performance of broilers. Poult. Sci. 72 (Suppl. 1):87. (Abstr.).

Martel, A., L. A. Devriese, K. Cauwerts, K. De Gaussem, A. Decostere, and F. Haesebrouck. 2004. Susceptibility of *Clostridium perfringens* strains from broiler chickens to antibiotics and anticoccidials. Avian Pathol. 33:3–7.

McBain, A. J., and G. T. MacFarlane. 2001. Modulation of genotoxic enzyme activities by non-digestible oligosaccharide metabolism in *invitro* human gut bacterial ecosystems. J. Med. Microbiol. 50:833–842.

McClane, B. A., 1994. *Clostridium perfringens* enterotoxin acts by producing small molecule permeability alterations in plasma membrane. Toxicology 87:43–67.

McDonel, J. L., and B. A. McClane.1979. Binding vs biological activity of *Clostridium perfringens* enterotoxin in vero cells. Biochem. Biophys. Res. Commun 87:497–504.

McReynolds, J. L., C. Waneck, J. Byrd, K. Genovese, S. Duke, and D. Nisbet. 2009. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. Poult. Sci. 88:2075–2080.

McDevitt, R. M., J. D. Brooker, T. Acamovic, and N. H. C. Sparks. 2006. Necrotic enteritis; a continuing challenge for the poultry industry. World's Poult. Sci. J. 62:221–247.

Mitsch, P. Z. E., K. Kohler, B. Gabler, C. Losa, R. Zimpernik. 2004. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chicken Poult. Sci. 83:669–675.

Nairn, M. E., and V. W. Bamford. 1967. Necrotic enteritis of broiler chickens in western Australia. Aust. Vet J 43:49–54.

Nisbet, D. J., D. E. Corrier, C. M. Scanlan, A. G. Hollister, R. C. Beier, and J. R. Deloach. 1993. Effect of a defined continuous flow-derived bacterial culture and dietary lactose on *Salmonella typhimurium* colonisation in broiler chickens. Avian Dis. 37:1017-1025.

Ouellette, A. J., and M. E. Selsted. 1996. Paneth cell defensins: endogenous peptide components of intestinal host defense. FASEB 10:1280–1289.

Parish, W. E. 1961. Necrotic enteritis in the fowl. I. Histopathology of the disease and isolation of a strain of *Clostridium welchii*. J. Comp. Path. 71:377–393.

Pascual, M., M. Hugas, J. I. Badiola, J. M. Monfort, and, M. Garriga. 1999. *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. Appl. Environ. Microb. 65:4981– 4986.

Pedersen, K. L. B., O. E. Heuer, M. A. Danilo, L. Wong, and B. Nauerby. 2008. Reproducable infection model in broiler chicken. Avian Dis. 52:34–39.

Petit, L, M. Gibert, and R. P. Michel. 1999. *Clostridium perfringens*: toxinotype and genotype. Trends Microbiol. 7:104–110.

Porter, R. E. 1998. Bacterial enteritides of poultry. Poult. Sci. 77:1159–1165.

Randall, C. J., H. Stevens, J. B. Walsby, J. B, and W. L. G. Ashton. 1983. Liver abnormality in broiler carcasses. Vet. Rec. 112: 159.

Riddell, C., and X. Kong. 1992. The influence of diet on necrotic enteritis in broiler chickens. Avian Dis. 36:499–503.

Robertson, S. L., J. G. Smedley, U. Singh, G. Chakrabarti, C. M. Van Itallie, J. M. Anderson, and B. A. McClane. 2007. Compositional and stoichiometric analysis of *Clostridium perfringens* enterotoxin complexes in Caco-2 cells and claudin 4 fibroblast transfectants. Cell Microbiol. 9:2734–55.

Sarker, M. R., R. J. Carman, and B. A. McClane. 1999. Inactivation of the gene (cpe) encoding *Clostridium perfringens* enterotoxin eliminates the ability of two cpe

positive *Clostridium perfringens* type A human gastrointestinal disease isolates to affect rabbit ileal loops. Microbiol. 33:946–958.

Savage, T. F., and E. I. Zakrewska. 1996. The performance of male turkeys fed a starter diet containing mannanoligosaccharide supplementation (Biomos) from day-old to eight weeks of age. Pg 47–54. Proceedings Alltech's 12th Annual Symposium on the Biotechnological Feed industry.

Shane, S. M., J. E. Gyimah, K. S. Harrington, and T. G. Snider.1985. Etiology and pathogenesis of necrotic enteritis. Vet. Res. Comm. 4:269–287.

Shin, Y. W., J. G. Kim, and K. Y. Whang. 2005. Effect of supplemental mixed yeast culture and antibiotics on growth performance of weaned pigs. J. Anim. Sci. 83(Suppl. 1):34.

Siragusa, G. R, G. J. Haas, P. D. Matthews, R. J. Smith, R. J. Buhr, N. M. Dale, and M. G. Wise. 2008. Antimicrobial activity of lupulone against *Clostridium perfringens* in the chicken intestinal tract jejunum and caecum J. Antimicrobiol. Chem. 61:853–858.

Songer, J. G. 1996. *Clostridial* enteric diseases of domestic animals. Clin Microbiol Rev. 9:216–34.

Specian, D., and M. Oliver. 1991. Functional biology of intestinal goblet cells. Am. J. Physiol. 260:183–193.

Stringfellow, K., J. McReynolds, J. Lee, J. Byrd, D. Nisbet, and M. Farnell.2009. Effect of bismuth citrate, lactose, and organic acid on necrotic enteritis in broilers Poult. Sci. 88:2280–2284.

Stutz, M. W., and. G. C. Lawton. 1984. Effect of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens* and the ideal weight of broiler chicks. Poult. Sci. 63:2036–2042.

Takeda T, Fukata T, Miyamoto T, Sasai K, Baba E, and Arakawa A. 1995. The effects of dietary lactose and rye on cecal colonization of *Clostridium perfringens* in chicks. Avian Dis. 39:375–381.

Tech Ross. 1999. Necrotic entritis and associated conditions in broiler chicken. World's Poult. Sci. 15:44–47.

Truscott, R. B., and F. Al-Sheikhly. 1977. Reproduction and treatment of necrotic enteritis in broilers. Am. J. Vet. Res. 38:857–861.

United States Feed Additive Compendium 2010. Miller publishing company.

Van Der Sluis, W. 2000. *Clostridial* enteritis is an often underestimated problem. World Poult. 16:42–43.

Wages, D. P., and K. Opengart. 2000. Necrotic enteritis. In: Diseases of poultry, 11th ed. Y.M. Saif, ed. Iowa State Press, Ames, IA. 781–785.

Waldenstedt, L., K. Elwinger, A. Lunden, P. Thebo, M. R. Bedford, and A. Uggla. 2000. Intestinal digesta viscosity decreases during coccidial infection in broilers. Br. Poult. Sci. 41:459–464.

Wilkie, D. C., A. G. Van Kessel, L. J. White, B. Laarveld, and M. D. Drew. 2005. Dietary amino acids affect intestinal *Clostridium perfringens* populations in broiler chickens. Can. J. Anim. Sci. 85:185–193.

Williams, R. B. 2002. Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathol. 31:317–53.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159–80.

Williams, R. B, R. N. Marshall, R. M. La Ragione, and J. Catchpole. 2003. A new method for the experimental production of necrotic enteritis and its use for studies on the relationships between necrotic enteritis, coccidiosis and anticoccidial vaccination of chickens. Parasitol. Res. 90:19–26.

Zhu, J., R.J. Meinersmann, K.L. Heitt, and D.L. Evans. 1999. Apoptotic effect of outer-membrane proteins from *Campylobacter jejuni* on chicken lymphocytes. Curr. Microbiol. 38:244-249.

Zimmer, M., N. Vukov, S. Scherer, and M. J. Loessner. 2002. The murein hydrolase of the bacteriophage f3626 dual lysis system is active against all tested *Clostridium perfringens* strains. Appl. Environ. Microb 68:5311–5317.

III. EXPERIMENT 1: EVALUATION OF THE EFFICACY OF NUPRO®-YEAST EXTRACT IN REDUCING INTESTINAL *CLOSTRIDIUM PERFRINGENS* LEVELS IN BROILER CHICKENS (BATTERY TRIAL)

3.1. Abstract

The etiological agent of Necrotic enteritis (NE) is *Clostridium perfringens* (CP). Traditionally, NE is controlled with in-feed antibiotics. However, increasing consumer demand for drug-free poultry has fostered the search for non-antibiotic alternatives. Yeast extracts contain nucleotides that are immunomodulatory and also essential for cellular functions. An experiment was conducted to evaluate the efficacy of NuPro®-yeast extract in reducing intestinal CP levels in broiler chickens. One hundred and ninety two day-old male broiler chicks were obtained, and randomly assigned to one of 6 treatments in a battery cage trial. Treatment 1 (CX) consisted of chicks fed corn-soybean meal basal diet (BD) without added bacitracin methylene disalicylate (BMD) or NuPro®. Treatment 2 (CM) consisted of chicks fed BD into which BMD was added at 0.055 g/kg. Treatment 3 (CN) consisted of chicks fed BD supplemented with NuPro® at 2% level for the first 10 days of experiment and supplemented with BD for the remainder of the trial. Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks that were challenged with 3 mL of CP inoculum (~10^7 cfu/mL) on days 14, 15, and 16 of experiment, and fed diets similar to treatments 1, 2, and 3, respectively. On days 1 and 7 post-challenge, intestinal CP levels,

lesion scores, and alkaline phosphatase activity (ALP) were assessed. On day 1 post-challenge, CP level in PM treatment (2.09 $\log_{10}$ cfu/g) was lower ($P < 0.05$) compared to the PX treatment (4.71 $\log_{10}$ cfu/g), but similar to PN treatment (2.98 $\log_{10}$ cfu/g). A similar trend was observed on day 7 post-challenge. NuPro® supplementation enhanced ALP activity ($P < 0.05$) in CP-challenged chicks, and appeared to reduce intestinal lesion scores. Although dietary supplementation of NuPro® in PN treatment reduced CP levels by 1.73 $\log_{10}$ cfu/g and 0.68 $\log_{10}$ cfu/g compared to PX treatment on day 1 and day 7 post-challenge, respectively, these reductions were not significant. Extending the period of NuPro® supplementation beyond the first 10 days of life should be considered for achieving significant reduction in intestinal CP levels.

3.2. Introduction

Necrotic Enteritis (NE) is an enteric disease of poultry that is caused by *Clostridium perfringens* (CP) Type A (McDevitt *et al*., 2006). Because of the gradual withdrawal of in-feed antibiotics that are typically used to control enteric (intestinal) diseases, NE has re-emerged as a challenge to the broiler industry (Hofacre *et al*., 1998; Young and Craig, 2001). Furthermore, the magnitude of economic loss resulting from NE (in terms of reduced bird performance and mortality) makes NE one of the most costly diseases associated with commercial broiler production (Beltran-Alcrudo *et al*., 2008). Estimates indicate that up to 37% of broilers grown in North America are affected by NE (Annett *et al*., 2002).

In poultry, NE is usually noticed 2-6 weeks after hatching (Fukata *et al*., 1991). The disease is propagated through horizontal (feed or litter contaminated with CP spores)

and vertical (from parents to progeny) modes of transmission (Heier *et al.*, 2001; Williams, 2002). Disease onset is accelerated by predisposing factors such as pre-existing damage to the intestinal epithelium by coccidia (*Eimeria* spp.), high dietary levels of certain cereals and fish meal, disturbance to the normal intestinal flora, overcrowding, and a variety of environmental (management and climatic) conditions (Hofacre, 2001; McDevitt *et al.*, 2006; Beltran-Alcrudo *et al*., 2008). Existence of favorable predisposing conditions in the intestine favors proliferation of CP and toxin production in the jejunum and ileum of the small intestine (Williams, 2005). CP can increase from levels normally seen in healthy birds (approximately 10^4 cfu/g of digesta) to about $10^7 – 10^9$ cfu/g of digesta (Williams, 2005; McDevitt *et al.*, 2006). Toxins produced by CP are known to induce mucosal damage (necrosis) in the sheep intestine (Fernandez-Miyakawa and Uzal, 2005). Damage to the intestinal mucosa results in concomitant loss of brush border enzymes (such as Alkaline phosphatase (ALP and maltase) and transport proteins that are responsible for the final stages of digestion of macromolecules and nutrient transport, respectively (Jeurissen *et al*., 2002; Fasina *et al*., 2007).

Depending on the number of CP cells in the intestine, NE can manifest as acute (clinical) or chronic (subclinical). Birds suffering from acute NE typically harbor CP levels of about $10^7 – 10^9$ cfu/g of digesta, and demonstrate symptoms characterized by diarrhea, necrotic intestinal lesions, depression in growth rate and feed efficiency, and high mortality (up to 40%; Long *et al*., 1974; Ross Tech, 1999; McDevitt *et al*., 2006). The chronic form of the disease is subtle in that it affects the welfare and productivity of birds in a manner that is difficult to measure until processing when high numbers of

carcasses and livers are condemned for low weight and cholangiohepatitis, (Stutz and Lawton, 1984., Lovland and Kaldhusdal, 1999).

There are several non-antibiotic strategies that have shown efficacy in controlling NE. Some of these include the administration of competitive exclusion (CE) products, probiotics (Elwinger *et al*., 1992; Dahiya *et al*., 2006), prebiotics (Branton *et al*., 1997), functional feed additives (yeast products, organic acids, enzymes, and essential oils), and dietary manipulation strategies (Jackson *et al*., 2003; Engberg *et al*., 2004; Mitsch *et al*., 2004). Dietary manipulation strategies such as substituting animal protein (fish meal) with plant protein, and replacing ground wheat in the diet with whole wheat have proven to be beneficial to preventing NE (Engberg *et al*., 2004; Dahiya *et al*., 2006). Hofacre *et al*. (2003) conducted a study in which day-old broiler chicks were fed a basal diet or subjected to various treatment regimens, which included the administration of BMD, and administration of non-antibiotic feed additives (such as lactic acid bacterial CE culture and mannan-oligosaccharide). Between day 15 and 20 of the study, broilers in each treatment were either subjected to experimental induction of NE, or kept disease-free. Results of which indicated that broilers treated with a defined lactic acid CE product at one day of age and fed a diet containing mannan-oligosaccharide at 0.002g/kg of feed, had the lowest mortality (30%) from NE, while the untreated broilers had a mortality of 60%. So far, all alternative (non-antibiotic) control strategies evaluated have furnished only partial protection against NE. Thus, it remains a priority for poultry scientists to identify a fully effective non-antibiotic supplement (or a suitable combination) that can completely replace the use of antibiotics for the control of CP and associated diseases.

Yeast extract is a non-antibiotic functional product that is naturally obtained from yeast strains such as *Saccharomyces cerevisiae* and *Kloeckera apiculata* (Owens and McCracken, 2007). Although the composition of yeast extracts are variable, they essentially contain nucleotides and β-glucans which are immunostimulatory and possibly antimicrobial (Savage and Zakerwska, 1996; Santos *et al*., 2007). Dietary supplementation of a yeast culture into broiler diets at 2.5 g/kg diet from day-old to 42 days resulted in a significant ($P < 0.05$) increase in serum lysozyme activity, duodenal secretory IgA concentrations, and growth performance (Gao *et al*., 2008). In another study, yeast culture was found to activate human natural killer cells and lymphocytes *in vitro* (Jensen *et al*., 2008). Interestingly, Line *et al*. (1997) observed that supplementing dried yeast at 10% level of the diet of 6 wk old broiler chickens for 60 h prior to being subjected to transportation stress reduced intestinal colonization by *Salmonella* and *Campylobacter*. Information is scanty or non-existent regarding the effect of dietary yeast extract supplementation on intestinal CP colonization of broiler chickens.

The goal of this study is to evaluate the efficacy of NuPro®-yeast extract supplementation in reducing clinical signs associated with CP and NE disease.

3.3. Materials and Methods

All the procedures used in this study were approved by the Auburn University Institutional Animal Care and Use Committee.

Experimental Design

One hundred and ninety two day-old male broiler chicks (Cobb x Cobb) were obtained from a commercial hatchery, and transported to the Auburn University Poultry Research and Teaching Farm. Upon arrival at the farm, the chicks were weighed, and randomly assigned to one of six dietary treatments (Table 3.2) in a completely randomized design. Treatment 1 (CX - control) consisted of chicks not challenged with CP and fed corn-soybean meal (SBM) diet with no BMD or NuPro® added. Treatment 2 (CM) consisted of chicks not challenged with CP and fed corn-SBM basal diet into which BMD was added at 0.055 g/kg. Treatment 3 (CN) consisted of chicks not challenged with CP and fed corn-soybean meal basal diet supplemented with NuPro® at 2% level for the first 10 days of experiment as recommended by manufacturer (Alltech Inc., Nicholasville, KY). Treatment 4 (PX), treatment 5 (PM), and treatment 6 (PN) consisted of chicks that were challenged with 3 mL of CP inoculum (10^7 cfu/mL) on three consecutive days (days 14, 15, and 16) during the experiment. Diets fed to chicks in PX, PM, and PN were similar to diets fed to chicks in CX, CM, and CN treatments, respectively. Each treatment consisted of 4 replicate pens with 8 chicks per pen. Experimental diets (Table 3.1) were formulated to meet the recommendations of the National Research Council (1994), and chicks were allowed *ad-libitum* access to feed and water throughout the experiment. Chicks were placed in Petersime raised wire batteries maintained at $30^oC \pm 2^oC$ during the first week, after which temperature was reduced weekly by 2^oC. Duration of experiment was 25 days.

Challenge Model and Preparation of CP Inoculum

The challenge model used in this experiment was a coccidia-free model designed as a modification of the models described by McReynolds *et al*. (2004) and Siragusa *et al*. (2008)**.** The three CP (type A) strains used for the challenge in this study were obtained from commercial flocks having NE in different geographical locations Texas, Virginia, and Georgia (McReynolds *et al*., 2004). To prepare the CP inoculum, each CP strain was streaked on 5% sheep's blood agar (Becton, Dickinson and Company, Sparks, MD) and incubated at 37°C for 24 hours inside anaerobic jars that utilized gas packs to generate anaerobic conditions (O_2 < 2% ; CO_2 = 9 – 13%; Mitsubishi Gas Chemical Company, Inc., Japan). Isolated white colonies with double zone of hemolysis obtained were inoculated into fluid thioglycolate broth (FTG, Oxoid Ltd, Hampshire, England) and incubated anaerobically at 37°C for 24 hours. A loop full of the FTG broth culture was streaked on Tryptose Sulfite Cycloserine Agar (TSC agar; Oxoid Ltd, Hampshire, England) and incubated anaerobically at 37°C for 24 hours. Black presumptive CP colonies were transferred into FTG broth and incubated anaerobically at 37°C for 18 hours. Thereafter, a cocktail of the three CP strains was prepared by mixing equal volumes of the FTG culture of each strain, resulting in a composite CP inoculum used for chick challenge. The concentration of CP cells in the inoculum was estimated spectrophotometrically at 600 nm with the aid of a standard curve. Actual CP concentration in the inoculum was confirmed by plating on TSC agar plates, incubating the plates anaerobically at 37°C for 18 hours, and counting the numbers of black presumptive CP colonies. Confirmation of colonies as CP was performed using the Rapid ID 32 A test kit (BioMerieux, Durham, NC).

On days 14, 15 and 16 of experiment, a fresh composite CP inoculum was prepared daily, after which a round tipped animal feeding needle (15 G, 78 mm; Solomon Scientific, PA, USA) attached to a repeating syringe (Popper & Sons, Inc., New Hyde Park, NY) was used to orally deliver 3 mL of the inoculum into the crop of each chick in the PX, PM, and PN treatments. Concentration of CP in the inoculum for each day was 7.3×10^7, 8.5×10^7, and 8.2×10^7 cfu/mL on days 14, 15, and 16, respectively. The chicks in the control treatments (CX, CM, and CN) were orally gavaged daily (on days 14, 15, and 16) with 3 mL of freshly prepared sterile FTG broth.

Sampling for Intestinal contents, Tissue Samples, Lesion Scoring and Spleen Weight

Intestinal sampling to determine the concentration of CP in intestinal contents was performed on days 13 (one day pre-challenge), 17 (one day post-challenge), and 23 (7 days post-challenge) of experiment. On each day, two chicks were randomly removed from each pen (totaling 8 chicks per treatment) and euthanized by CO_2 asphyxiation. Thereafter, the intestine of each chick was aseptically excised, and a 15 cm cranial to Meckel's diverticulum section was removed and kept on ice for subsequent enumeration of CP on TSC. Next, a 1 cm portion of the proximal jejunum was taken, washed twice in PBS (1x) to remove the intestinal digesta, snap frozen in liquid N_2, and kept at -70^oC until time to determine alkaline phosphatase (ALP) activity. Lastly, the duodenum was assessed for intestinal lesions using a scale described by (Prescott *et al.*, 1978). Briefly, lesions were scored on a 0 - 4 scale where 0 - no apparent lesions, 1 - thin friable small intestine, 2 - focal necrosis, ulceration or both, 3 - patchy necrosis, and 4 - severe

extensive mucosal necrosis. The spleen was excised aseptically and the weight was recorded.

Determination of Intestinal CP Concentration

From each intestinal section collected for CP enumeration, approximately 5 g of intestinal content was weighed, transferred to 10 mL of anaerobic FTG broth, and stomached for 30 seconds (Stomacher 400 Circulator, Seward Limited, London, UK). One mL of stomached digesta was transferred to 9 mL of anaerobic FTG broth, and serially diluted into FTG (ten-fold dilutions). The dilutions were then plated on TSC agar, and incubated anaerobically at 37°C for 24 hours. The number of characteristic black colonies was then counted.

Determination of ALP Activity

Previously frozen jejunal tissue samples were thawed on ice and 350 mg of each tissue was homogenized in 7 mL of 50 m*M* sodium phosphate buffer (Fisher Scientific, NJ, USA) containing 0.5% Triton X-100 (Fisher Biotech, NJ, USA). Resulting homogenate was then centrifuged at 10,000X G (Sorvall Legend 23R) for 15 minutes, and the supernatant was collected for estimation of protein (Quick Start™ Bradford Protein Assay; Bio-Rad, Hercules, CA) and ALP activity (Quantichrom™ Alkaline phosphatase assay kit; BioAssay Systems, Hayward, CA). The assay utilized stable *p*-nitrophenol phosphate as substrate, and ALP activity was expressed as IU/mg protein.

Performance Evaluation

Body weight and feed conversion efficiency (FCE – calculated as feed-to-gain ratio) were recorded on day 7, 14, and 21 of the experiment. Mortality was recorded daily.

Statistical Analysis

Using the General Linear Models (GLM) procedure of SAS (SAS Institute, 2004) intestinal CP concentration was transformed to $\log_{10}$ values and were subjected to one-way ANOVA along with ALP activity, lesion scores, relative spleen weight and growth performance data (body weight and FCE). Significant differences among means were determined using the Duncan option of the GLM procedure as a post hoc test (Waller and Duncan, 1969; SAS Institute Inc., 2004). Mortality data was subjected to arcsine square root transformation, and subsequently analyzed by ANOVA, and Tukey test was applied for means separation (SAS Institute, 2004). Data are presented as means ± SEM. Statements of statistical significance were based upon $P < 0.05$.

3.4. Results and Discussion

Intestinal CP Concentration

Intestinal CP concentrations are presented in Table 3.3. In all treatments, baseline CP concentration determined at one day pre-challenge (day 13 of experiment) was negligible and therefore not reported. This implies that all chicks in the experiment probably had a similar concentration of intestinal CP at the beginning of the experiment, prior to CP challenge.

On day 1 post-challenge, all unchallenged treatments (CX, CM, and CN) had CP concentrations that were lower ($P < 0.05$) than those of PX and PN treatments. Among the challenged treatments (PX, PM, PN), intestinal CP level for PM treatment (2.09 $\log_{10}$ cfu/g) was lower ($P < 0.05$) compared to PX treatment (4.71 $\log_{10}$ cfu/g), but similar to levels for PN treatment (2.98 $\log_{10}$ cfu/g; Table 3.3). The reduced CP infection in PM treatment could be due to the bactericidal action of BMD present in the feed. BMD is active against gram-positive bacteria, and is commonly included in commercial poultry diets for the control of CP-induced NE (Sims *et al*., 2004). Although there was no significant difference between the PN and PX, there was a 1.73 $\log_{10}$ cfu/g reduction between them. Perhaps increasing the number of chicks sampled could have reduced the variability in the data and enhanced significance of results. The level of CP in the unmedicated challenged treatment (PX) was comparable to the levels reported by other authors. McReynolds *et al*. (2009) and Stringfellow *et al*. (2009) reported a CP level of 4.96 $\log_{10}$ cfu/g and 3.59 ± 2.2 $\log_{10}$ cfu/g, respectively, in broiler chicks challenged with CP and fed an unmedicated control diet.

On day 7 post-challenge, the trend of intestinal CP concentration was similar to that observed for day 1 post-challenge. The unchallenged treatments (CX, CM, and CN) had CP concentrations that were lower ($P < 0.05$) than those of PX and PN, confirming that the CP infection established by day 1 post-challenge was sustained till day 7 post-challenge. On comparing CP levels of unchallenged treatments (CX, CM, and CN) on day 1 post-challenge with day 7 post-challenge, it was observed that CP levels in these treatments on day 7 post-challenge (1.50 to 2.34 $\log_{10}$ cfu/g) was higher than their corresponding levels on day 1 post-challenge (0.26 $\log_{10}$ cfu/g; Table 3.3). The higher

CP levels in the unchallenged treatments on day 7 post-challenge was probably due to chicks picking up CP in their feed and feces. The level of CP recovered from the intestine of challenged chicks in this study is comparable to levels reported in literature for NE-infected chicks. For instance, the level of CP in PX treatment (unmedicated) on day 7 post-challenge (4.11 $\log_{10}$ cfu/g) was comparable to the level (4.98 $\log_{10}$ cfu/g) reported by Pedersen *et al*. (2008) in chicks sampled a week post-challenge and maintained on an antibiotic-free diet. Compared to PX treatment, BMD (fed to PM birds) and NuPro®-yeast extract (fed to PN birds) reduced intestinal CP levels by 1.08 and 0.68 $\log_{10}$ cfu/g, respectively.

Lesion scores, ALP Activity and Spleen Weight

The lesion scores of the chicks on day 1 post-challenge are presented in Figure 3.1. With the exception of PN treatment, birds challenged with CP (PX and PM treatments) had higher lesion scores ($P < 0.05$; from 1.4 to 1.9) compared to birds in unchallenged treatments (CX, CM, and CN). The lesion scores observed in this study for unmedicated challenged chicks (PX treatment) is comparable to levels reported in literature for NE infection. For instance, Brennan *et al*. (2003) reported a lesion score of 1.8 in chicks sampled day one post-challenge. The similarity in lesion scores between challenged PN and unchallenged CX and CM implies that NuPro® supplementation favored the reduction of NE lesions in the gut of broiler chickens. The exact mode of action of NuPro®-yeast extract in reducing intestinal CP-induced lesions is not clearly understood. *Saccharomyces cerevisiae* yeast increases cytokine production by macrophages (Adachi *et al*., 1994), and enhances in vitro proliferative response of human

lymphocytes (Darroch *et al*., 1994), NuPro®-yeast extract could also have stimulated the intestinal immune system by increasing intestinal IgA production as found in dogs (Swanson *et al*., 2002), rats (Kudoh *et al*., 1999), and chickens (Gao *et al*., 2008) fed yeast supplements. IgA binds to antigens (perhaps such as CP α-toxin) and prevents them from passing through the mucosal membrane and establishing infection and lesions (Kulkarni *et al*., 2010). The immunomodulatory effect of yeast is attributed to its constituent nucleotides, mannan oligosachharides, and β glucans (Newman, 1994; Lowry *et al*., 2005; Chae *et al*., 2006; Santos *et al*., 2007). To explore this, further studies need to be performed on investigating the mechanism(s) of the immunomodulatory activity of yeast and yeast products in poultry. Spleen is one of the secondary lymphoid tissue associated with gut activity and forms the major constituent of the immune system (Yegani and Korver, 2008). Yeast cell components acts as a non pathogenic antigen and stimulates development of lymphoid tissues (Ferket et.al., 2002). Also, microbial antigens stimulates the development of lymphoid tissues (Pabst *et al*., 1998). Guo et al. (2003) and Zhang et al. (2008) observed an increase of spleen relative weight in broilers fed diets supplemented with 40 or 50 mg of beta glucans / kg of feed. In the current study NuPro® did not have any effect on relative spleen weight (Figure 3.4). In agreement with our findings Morales-Lopez *et al* 2009., did not find an effect on relative spleen weight when fed dietary mannoproteins at 95 mg /kg of feed and beta glucans at 145 mg /kg of feed in broilers.

The effect of NuPro® yeast extract supplementation on ALP activity is presented in figure 3.2 and 3.3. In the intestine, the expression of ALP enzyme activity increases as

enterocytes mature and move upwards toward the tip of the villi (Uni, 1999). Hence an increased ALP activity indicates a higher number of functional and mature enterocytes in the gut (Fasina *et al*., 2004). On day 1 post-challenge, jejunal ALP activity in PN treatment was higher ($P < 0.05$) than ALP activity in other treatments. Yeast supplements are known to promote intestinal mucosal development in broiler chickens (Santin *et al*., 2001; Zhang *et al*., 2005). However by day 7 post challenge the beneficial effect of Nupro® in enhancing ALP activity was no longer apparent.

Growth Performance and Mortality

Effect of Nupro® on growth performance parameters is presented in Table 3.4. In the present study, NuPro® had no effect ($P > 0.05$) on the performance parameters evaluated. The presented results were consistent with some previous work, while contradictory to others. It has been noted that yeast or the yeast cell components frequently did not produce consistent results on growth performance parameters. In some studies, yeast was found to improve feed utilization and body weight in broiler chickens (Madrigal *et al*., 1993) and turkey poults (Bradley *et al*., 1994). On the contrary, Morales-López *et al*. (2009) evaluated the effect of various yeast cell components on growth performance parameters in broiler chicks but found no effect. The FCR values by day 21 obtained in the present study for the yeast extract supplemented treatment (1.359) was comparable to those obtained by Morales-López *et al*. (2009,1.452) in which the birds were fed dietary beta glucans at 145 mg /kg of feed.

Total mortality in this experiment was 2.6%. In an untreated NE-infected flock, mortality rate can range from 10 to 40% (Ross Tech, 1999). However, in this study, no difference ($P > 0.05$) was observed in mortality rate between unchallenged (CX, CM, and CN) and challenged (PX, PM, and PN) treatments. This observation is consistent with a condition of sub-clinical NE in which mortality rarely occurs (McDevitt *et al.*, 2006).

In summary, we evaluated the efficacy of NuPro®-yeast extract in reducing intestinal CP levels in broiler chicks. To conduct this investigation, we experimentally induced CP infection and sub-clinical NE in the intestine of broiler chickens. One and seven days, post-infection CP levels, lesion scores, and ALP activity in the intestine of experimental chicks were assessed. Growth performance parameters were also evaluated. Results showed that supplementing NuPro®-yeast extract into the diet of broiler chicks during the first 10 days after hatch showed some potential to reduce intestinal CP levels. Of more interest is that, compared to PX, NuPro® decreased intestinal CP levels by 1.73 $\log_{10}$ cfu/g and 0.68 $\log_{10}$ cfu/g at 1- and 7-days post-challenge without causing any adverse effect on chick growth performance. Supplementing NuPro® for periods longer than the first 10 days of life may achieve reductions of higher (or significant) magnitude in CP levels in the broiler intestine.

3.5. Refrences

Adachi, Y., M. Okazaki, N. Ohno, and T. Yadomae. 1994. Enhancement of cytokine production by macrophages stimulated with (1-3)-*β*-D-glucan, grifolan (GRN), isolated from *Grifola frondrosa*. Biol. Phar. Bull. 17:1554–1560.

Annett, C. B., J. R. Viste, M. Chirino-Trejo, H. L. Classen, D. M. Middleton, and E. Smiko. 2002. Necrotic enteritis: effects of barley, wheat and corn diets on proliferation of *Clostridium perfringens* type A. Avian Pathol. 31:599–602.

Beltran-Alcrudo, D., C. Cardona, L. McLellan, N. Reimers, and B. Charlton. 2008. A persistent outbreak of ulcerative enteritis in Bobwhite Quail (Colinus virginianus). Avian Dis. 52:531–536.

Bradley, G. L., T. F. Savage, and K. I. Timm, 1994. The effects of supplementing diets with *Saccharomyces cerevisiae* var. *boulardii* on male poult performance and ileal morphology. Poult. Sci. 73:1766–1770.

Branton, S. L., B. D. Lott, L. W. Deaton, W. R. Maslin, F. W. Austin, L. M. Pote, R. W. Keirs, M. A. Latour, and E. J. Day. 1997. The effect of added complex carbohydrates or added dietary fibers on necrotic enteritis lesions in broiler chicken. Poult. Sci. 76:24–28.

Brennan, J., J. Skinner, D. A. Barnum, and J. Wilson. 2003. The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poult. Sci. 82:360–363.

Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, and T. W. Hahn. 2006. Effects of supplementation of β-glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291–298.

Darroch, C. J., S. E. Christmas, and R. M. R. Barnes. 1994. In vitro human lymphocyte proliferative responses to a glycoprotein of the yeast *Saccharomyces cerevisiae*. Immunology 81:247–252.

Dahiya, J.P., D.C. Wilkie, A.G. van Kessel, and M.D. Drew. 2006. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim. Feed Sci. Tech. 129:60-88.

Elwinger. K. C., S. F. Berndtson, O. Fossum, B. Teglof, and B. Bagstrom. 1992. Factors affecting the incidence of necrotic enteritis, cecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. Acta. Vet. Scand 33:369–378.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925–938.

Fasina, Y. O., J. D. Garlich, H. L. Classen, P. R. Ferket, G. B. Havenstein, J. L. Grimes, M. A. Qureshi, and V. L. Christensen. 2004. Response of turkey poults to soybean lectin levels typically encountered in commercial diets. 1. Effect on growth and nutrient digestibility. Poult. Sci. 83: 1559–1571.

Fasina, Y.O., E. T. Moran, C. M. Ashwell, D.E. Conner, M. Leslie, and S.R. McKee. 2007. Effect of dietary gelatin supplementation on the expression of selected enterocyte Genes, Intestinal Development and Early Chick Performance. Int. J. Poult. Sci. 12:944-951.

Ferket, P. R., C. W. Parks, and J. L. Grimes. 2002. Benefits of dietary antibiotic and mannanoligosaccharide supplementation for poultry. In Proc. Multi-State Poultry Feeding and Nutrition Conference, Indianapolis, IN. http://ag.ansc.purdue.edu/poultry/multistate/Proceedings.MultiState%20MeetingFerket.pdf Accessed June 2006.

Fernandez-Miyakawa, M. E., and F. A. Uzal. 2005. Morphologic and physiologic changes induced by *Clostridium perfringens* type A alpha toxin in the intestine of sheep. Am. J. Vet. Res. 66:251-255.

Fukata, T., Y Hadate, E. Baba, and A. Arakawa. 1991. Influence of bacteria on *Clostridium perfringens* infection in young chickens. Avian Dis. 35:224–227.

Guo, Y., R. A. Ali, and A. M. Qureshi. 2003. The influence of β-glucan on immune response in broiler chicks. Immunopharmacol. Immunotoxicol. 25:461–472.

Gao, J., H. J. Zhang, S. H. Yu, S. G. Wu, I. Yoon, J. Quigley, Y. P. Gao, and G. H. Qi. 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poult. Sci. 87: 1377–1384.

Heier, B. T., A. Lovland, K. B. Soleim, M. Kaldhusdal, and J. Jarp. 2001. A field study of naturally occurring specific antibodies against *Clostridium perfringens* alpha toxin in Norwegian broiler flocks. Avian Dis. 45:724–732.

Hofacre, C. L. 2001. Necrotic enteritis, currently a billion dollar disease; is there anything new on the horizon? Science and Technology in the feed industry; Proceedings of Alltech's Seventeenth Annual Symposium. Lexington, KY. 79–86.

Hofacre, C. L., R. Froyman, B. Gautrias, B. George, M. A. Goodwin, and J. Brown.1998. Use of aviguard and other intestinal bioproducts in experimental *Clostridium perfringens*-associated necrotizing enteritis in broiler chickens. Avian Dis. 42: 579–584.

Hofacre, C. L., T. Beacorn, S. Collett, and G. Mathis. 2003. Using competitive exclusion, mannan-oligosaccharide and other intestinal products to control necrotic enteritis. J. Appl. Poult Res. 12:60–64.

Jackson, M. E., D. M. Anderson, H. Y. Hsiao, G. F. Mathis, and D.W. Fodge. 2003. Beneficial effect of beta mannase feed enzyme on performance of chicks challenged with *Eimeria* sp. and *Clostridium perfringens*. Avian Dis. 47:759–763.

Jensen, G. S., K. M. Patterson, and I. Yoon. 2008. Yeast culture has anti-inflammatory effects and specifically activates NK cells. Comp. Immunol. Microbiol. Infect. Dis. 6:487–500.

Jeurissen, S.H.M., F. Lewis, J.D. van der Klis, Z. Mroz, J.M.J. Rebel, and A.A.H.M. ter Huurne. 2002. Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. Curr. Issues Intest. Microbiol. 3:1-14.

Kudoh, K., J. Shimizu, A. Ishiyama, M. Wada, T. Takita, Y. Kanke, and S. Innami. 1999. Secretion and excretion of immunoglobulin A to cecum and feces differ with type of indigestible saccharides. J. Nutr. Sci. Vitaminol. (Tokyo) 45:173–181.

Kulkarni, R.R., V.R. Parreira, Y.F. Jiang, and J.F. Prescott. 2010. A live oral recombinant *Salmonella enterica* serovar Typhimurium vaccine expressing *Clostridium perfringens* antigens confers protection against necrotic enteritis in broiler chickens. Clin. Vac. Immunol. 17:205-214.

Line, E. J., J. S. Bailey, N. A. Cox, and J. S. Norman. 1997. Yeast treatment to reduce *Salmonella* and *Campylobacter* populations associated with broiler chickens subjected to transport stress. Poult. Sci. 76:1227–1231.

Long, J. R., J. R. Pettit, and D. A. Barnum. 1974. Necrotic Enteritis in Broiler Chickens II. Pathology and Proposed Pathogenesis. Can. J. Comp. Med. 38:467–474.

Lovland, A, and M. Kaldhusdal. 1999. Liver lesions seen at slaughter as an indicator of necrotic enteritis in broiler flocks. FEMS Immunol. Med. Microbiol. 24:345–351.

Lowry, V. K., M. B. Farnell, P. J. Ferro, C. L. Swaggerty, A. Bahl, and M. H. Kogut. 2005. Purified *β*-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar *Enteritidis*. Int. J. Food Microbiol. 98:309–318.

Madrigal, S. A., S. E. Watkins, J. T. Skinner, M. H. Adams, A. L. Waldroup, and P. W. Waldroup, 1993. Effect of an active yeast culture on performance of broilers. Poult. Sci. 72 (Suppl. 1):87.

McDevitt, R. M., J.D. Brooker, T. Acamovic, and N.H.C. Sparks. 2006. Necrotic enteritis; a continuing challenge for the poultry industry. World's Poult. Sci. J.62: 221–247.

McReynolds, J. L., J. A. Byrd, R. C. Anderson, R. W. Moore, T. S. Edrington, K. J. Genovese, T. L. Poole, L. F. Kubena, and D. J. Nisbet. 2004. Evaluation of immunosuppressants and dietary mechanisms in an experimental disease model for necrotic enteritis. Poult. Sci. 83:1948–1952.

McReynolds, J., C. Waneck , J. Byrd , K. Genovese , S. Duke , and D. Nisbet. 2009. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. Poult. Sci. 88:2075–2080.

Mitsch, P. Z. E., K. Kohler, B. Gabler, C. Losa, R. Zimpernik. 2004. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chicken Poult. Sci. 83:669–675.

Morales-López, R., E. Auclair, F. García, E. Esteve-Garcia, and J. Brufau. 2009. Use of yeast cell walls; β-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. Poult. Sci. 88:601–607.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Newman, K. E. 1994. Mannan-oligosaccharides: Natural polymers with significant impact on the gastrointestinal microflora and the immune system. Biotechnology in the feed industry. Pages 167–174 In T. P. Lyons and K. A. Jaques, ed. Proc. Alltech's 10th Annual Sym. Nottingham University Press, Loughborough, Leics, UK.

Owens, B., and K. J. McCracken. 2007. A comparison of the effects of different yeast products and antibiotic on broiler performance. Br. Poult. Sci. 48:49–54.

Pabst, R., M. Geist, H. J. Rothkötter, and F. J. Fritz. 1998. Postnatal development and lymphocyte production of jejunal and ileal Peyer's patches in normal and gnotobiotic pigs. Immunol. 64:539–544.

Pedersen, K., L. Bjerrum, O. E. Heuer, D. M. A. Lo. Fo. Wong, and B. Nauerby. 2008. Reproducible infection model for *Clostridium perfringens* in broiler chickens. Avian Dis. 52:34–39.

Prescott, J. F., R. Sivendra, and D. A. Barnum. 1978. The use of bacitracin in the prevention and treatment of experimentally induced necrotic enteritis in the chicken. Can. Vet. J. 19:181–183.

Ross. Tech. 1999. Necrotic enteritis and associated conditions in broiler chicken. World Poultry. 15:44–47.

Santin, E., A. Maiorka, and M. Macari. 2001. Performance and intestinal mucosa development of broilers chickens fed diets containing *Saccharomyces cerevisiaes* cell wall. J. Appl. Poult. Res. 10:236–244.

SAS Institute. 2004. SAS/STAT User's Guide. Version 9.1 for Windows. SAS Inst. Inc., Cary, NC.

Savage, T. F., and E. I. Zakrewska. 1996. The performance of male turkeys fed a starter diet containing mannanoligosaccharide supplementation (Biomos) from day-old to eight weeks of age. Pg 47–54. Proceedings Alltech's 12th Annual Symposium on the Biotechnological Feed industry.

Sims, M. D., K. A. Dawson, K. E. Newman, P. Spring, and D. M. Hooge. 2004. Effects of dietary mannan Oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. Poult. Sci. 83:1148-1154.

Siragusa, G. R, G. J. Haas, P. D. Matthews, R. J. Smith, R. J. Buhr, N. M. Dale, and M. G. Wise. 2008. Antimicrobial activity of lupulone against *Clostridium perfringens* in the chicken intestinal tract jejunum and caecum J. Antimicrob. Chemoth. 61:853–858.

Solis de los Santos, F., A. M. Donoghue, M. B. Farnell, G. R. Huff, W. E. Huff, and D. J. Donoghue. 2007. Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune). Poult. Sci. 86:921–930.

Stringfellow, K., J. McReynolds , J. Lee , J. Byrd , D. Nisbet , and M. Farnell. 2009. Effect of bismuth citrate, lactose, and organic acid on necrotic enteritis in broilers. Poult. Sci. 88:2280–2284.

Stutz, M. W., and G. C. Lawton. 1984. Effect of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens* and the ideal weight of broiler chicks. Poult. Sci. 63:2036–2042.

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, H. P. Healy, K. A. Dawson, N. R. Merchen, and G. C. Fahey. 2002. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. J. Nutr. 132:980–989.

Uni, Z., 1999. Functional development of the small intestine: cellular and molecular aspects. Poult. Avian Biol. Rev.10:167–179.

Waller, R. A., and D. B. Duncan. 1969. A Bayes Rule for the symmetric multiple comparison problem. J. Am. Stat. Assoc. 64: 1484–1499.

Williams, R. B. 2002. Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathol. 31: 317–353.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159–180.

Yegani, M., and D. R. Korver. 2008. Factors affecting intestinal health in poultry Poult. Sci. 87:2052–2063.

Young, R., and A. Craig. 2001. The use and misuse of antibiotics in UK Agriculture – Part 3. Residues of dangerous drugs in intensively produced chicken meat and eggs. Bristol, UK: The Soil Association.

Zhang, B., Y. Guo, and Z. Wang. 2008. The modulation effect of β-1, 3/1, 6-glucan supplementation in the diet on performance and immunological response of broiler chickens. Asian-austalas. J. Anim. Sci. 21:237–244.

Zhang, A. W., B. D. Lee, S. K. Lee, K. W. Lee, G. H. An, K. B. Song, and C. H. Lee. 2005. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broilers chicks. Poult. Sci. 84:1015–102.

Table 3.1. Composition of experiment 1 diet

Item	Diets[1]		
Ingredients (% of diet)	Corn-SBM Only	Corn-SBM with BMD	Corn-SBM with NuPro®
Corn	54.6	54.6	54.6
Soybean meal	36.8	36.8	36.8
Poultry fat	3.70	3.70	3.70
Dicalcium phosphate	1.74	1.74	1.74
Limestone	1.68	1.68	1.68
DL-Methionine	0.20	0.20	0.20
Salt	0.40	0.40	0.40
Trace minerals[2]	0.25	0.25	0.25
Vitamins[3]	0.25	0.25	0.25
Bacitracin (Antibiotic, g/kg)	-	0.055	-
NuPro® (%)	-	-	2.00
Calculated analysis			
CP (%)	23.00	23.00	23.00
ME kcal/kg	3200	3200	3200
Calcium (%)	1.10	1.10	1.10
Available phosphorus (%)	0.13	0.13	0.13
Methionine (%)	0.54	0.54	0.54
Methionine + cystine (%)	0.90	0.90	0.90
Lysine (%)	1.25	1.25	1.25

[1]Diets include the corn soybean meal (SBM) basal, the corn-SBM basal diet supplemented with bacitracin methylene disalicylate (BMD) at 0.55 g/kg diet, and the corn-SBM basal supplemented with NuPro®-yeast extract at 2% level of the diet.

[2]Provides the following per kg of diet: iodine, 1mg; copper, 6 mg; manganese, 65 mg; cobalt, 0.2mg; iron, 55 mg; zinc, 55 mg.

[3]Provides the following per kg of diet: vitamin A, 8,000IU (retinyl palmitate); cholecalciferol, 2,000 IU; vitamin E (DL-α-tocopherol acetate), 8 IU; menadione, 2 mg; riboflavin, 5.5 mg; panthothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; vitamin $B_{12,}$ 0.02 mg; folic acid, 0.5 mg; thiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05mg; ethoxyquin, 125mg.

Table 3.2. Description of experimental treatments (experiment 1)

Treatment[1]	Challenge	Bacitracin	NuPro®
CX	-	-	-
CM	-	+	-
CN	-	-	+
PX	+	-	-
PM	+	+	-
PN	+	-	+

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (CN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, & 3 respectively.

Table 3.3. Effect of NuPro® supplementation on intestinal *C. perfringens* levels (experiment 1)

Treatment[1]	Log_{10} cfu/g	
	Day 1 Post challenge	Day 7 Post challenge
CX	0.26^{c}	2.10^{bc}
CM	0.26^{c}	1.50^{c}
CN	0.26^{c}	2.34^{bc}
PX	4.71^{a}	4.11^{a}
PM	2.09^{bc}	3.03^{ab}
PN	2.98^{ab}	3.43^{a}
SEM	0.607	0.361
P-value	0.0002	0.0001

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (CN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, & 3 respectively.

Table 3.4. Effect of NuPro® supplementation on broiler performance (experiment 1)

Treatment[1]	Body weight(g)			Feed conversion ratio			
	wk1	wk2	wk3	wk1	wk2	wk3	Cum
CX	165.5	452.3	1027.3	1.169	1.288	1.370	1.306
CM	165.1	445.0	979.6	1.117	1.325	1.400	1.326
CN	165.5	453.3	941.0	1.134	1.269	1.359	1.320
PX	154.9	451.0	933.8	1.188	1.227	1.372	1.294
PM	156.3	428.7	941.3	1.201	1.291	1.364	1.313
PN	167.3	455.0	944.9	1.131	1.314	1.322	1.286
SEM	4.276	12.927	36.943	0.040	0.029	0.048	0.023
P-value	0.2405	0.7116	0.4811	0.6181	0.2990	0.9215	0.8184

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (CN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, & 3 respectively.

[2] Cum = Cumulative of wk1, wk2, and wk3 FCR.

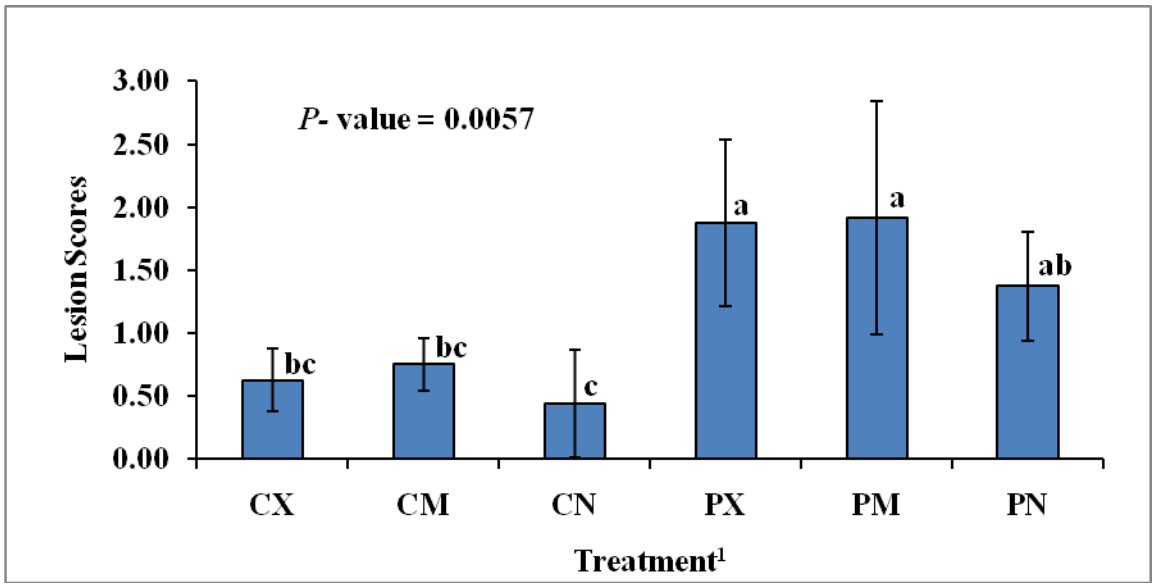

Figure 3.1. Effect of NuPro® supplementation on lesion scores at day 1 post challenge (experiment 1).

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (CN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks in treatments 1, 2, & 3 respectively.

[abc]Means bearing different superscript are significantly ($P < 0.05$) different.

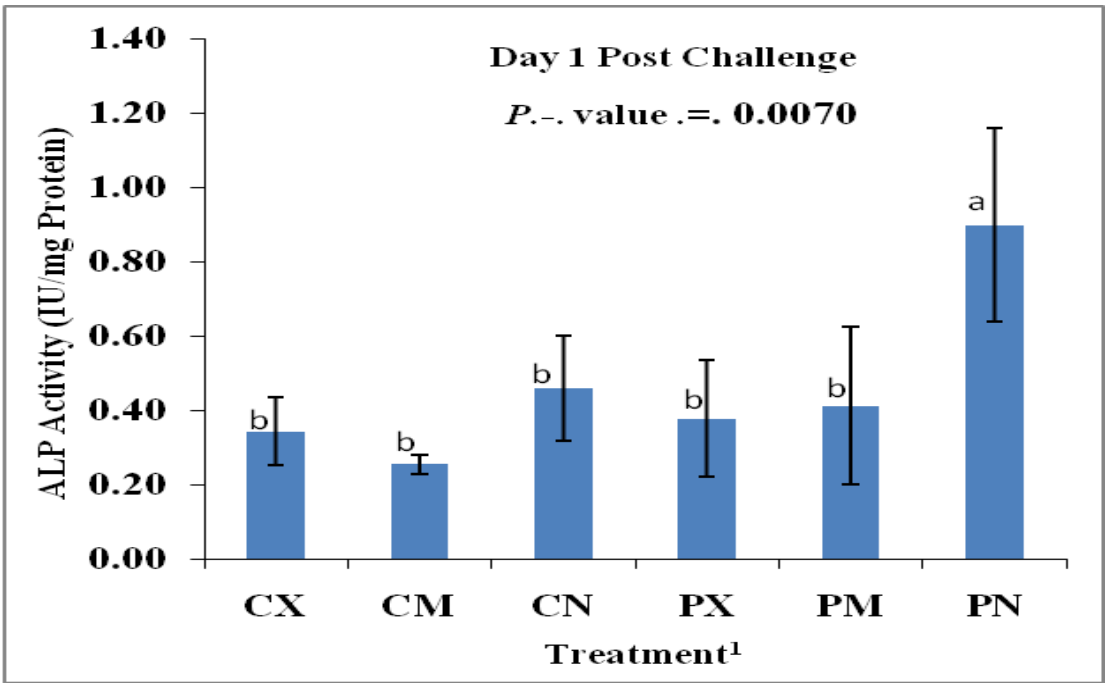

Figure 3.2. Effect of NuPro® supplementation on alkaline phosphatase activity at day 1 post challenge (experiment 1).

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (CN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks in treatments 1, 2, & 3 respectively.

[abc]Means bearing different superscript are significantly ($P < 0.05$) different.

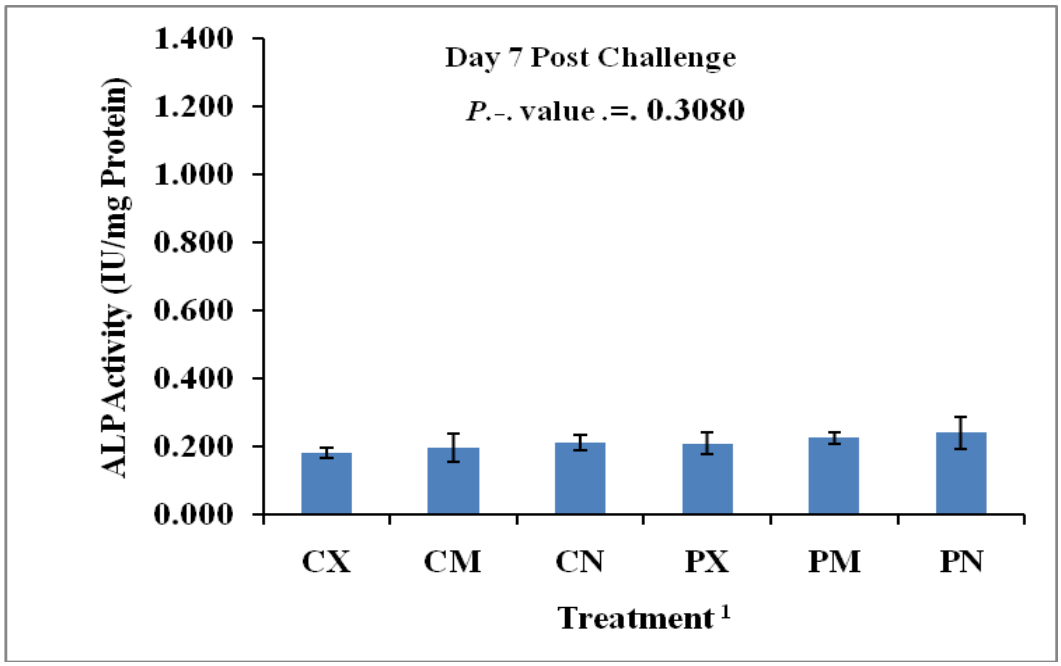

Figure 3.3. Effect of NuPro® supplementation on alkaline phosphatase activity at day 7 post challenge (experiment 1).

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (CN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks in treatments 1, 2, & 3 respectively.

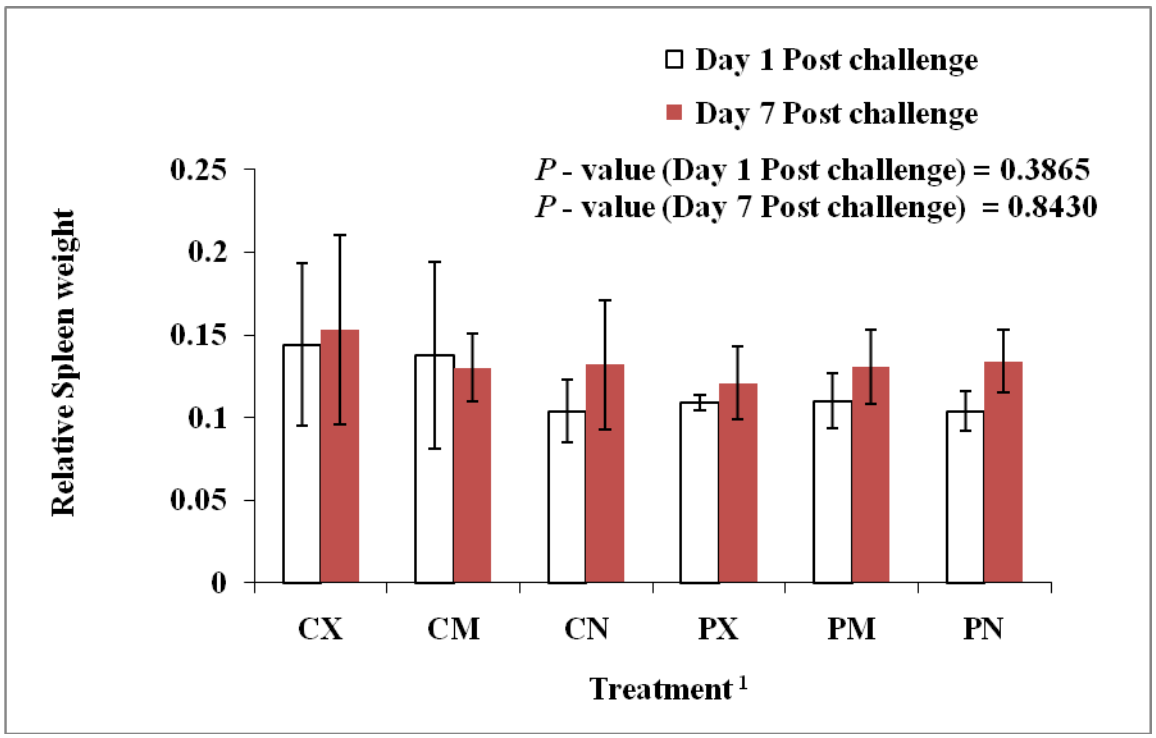

Figure 3.4. Effect of NuPro® supplementation on relative spleen weight at day 1 and 7 post challenge (experiment 1).

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (CN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatments 4 (PX), 5 (PM), and 6 (PN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks in treatments 1, 2, & 3 respectively.

IV. EXPERIMENT 1: EVALUATION OF THE EFFICACY OF NUPRO®-YEAST EXTRACT IN REDUCING INTESTINAL *CLOSTRIDIUM PERFRINGENS* LEVELS IN BROILER CHICKENS (FLOOR PEN TRIAL)

4.1. Abstract

Clostridium perfringens (CP) is the causative bacteria for necrotic enteritis (NE) in poultry. Yeast extract contain immunomodulatory nucleotides, and may therefore serve as a non-antibiotic feed additive for reducing intestinal CP in broilers. In a 42-day floor pen trial, the efficacy of NuPro® (a commercial yeast extract) in reducing intestinal CP levels in broiler chickens was evaluated. Eight hundred day old male broiler chicks were obtained from a commercial hatchery and randomly assigned to one of eight treatments. Treatment 1 (CX) consisted of chicks fed corn-soybean meal (SBM) diet without bacitracin methylene disalicylate (BMD) or NuPro® added. Treatment 2 (CM) consisted of chicks fed corn-SBM basal into which BMD was added at 0.055 g/kg. Treatment 3 (SN) consisted of chicks fed corn-SBM basal into which NuPro® was added at 2% level for the first 10 days of the experiment. Treatment 4 (LN) consisted of chicks fed corn-SBM basal into which NuPro® was added at 2% level throughout experiment. Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks fed diets similar to those given to CX, CM, SN and LN treatments respectively, and were challenged with 3.5 mL of CP inoculum (10^8 cfu/mL) on days 14, 15, and 16 of experiment. On days 1, 7, and 21 post-

challenge intestinal CP levels were assessed. Growth performance body weight (BW) FCR and percent uniformity was also assessed on days 21 and 42. Results showed that by day 21 post challenge, the NuPro®-containing diet fed throughout the experiment significantly reduced intestinal CP levels ($P < 0.05$) in PLN treatment by 1.5 $\log_{10}$ cfu/g compared to NuPro®-free PX treatment. On the other hand, the magnitude of intestinal CP reduction by PM (1.19 $\log_{10}$ cfu/g) and PSN (1.32 $\log_{10}$ cfu/g) was not significant. There was no significant difference in the performance parameters evaluated. NuPro®-induced reduction of CP in PLN treatment occurred without any adverse effect on bird performance. In conclusion, dietary supplementation of NuPro® effectively reduced intestinal CP during broiler production cycle.

4.2. Introduction

Antibiotics are added to poultry feed as supplements to serve two major purposes. To improve growth rate and feed efficiency of birds and as a prophylactic measure to prevent certain diseases that are of importance to the bird health and also from a food safety perspective (Solomons, 1978). Recently, due to increased consumer and governmental pressure to halt antibiotic addition in animal feed, several non antibiotic natural feed additives have been investigated. NE caused by CP is an important enteric disease of poultry which causes economic losses to poultry producers (Kaldhusdal and Lovland, 2000; McDevitt *et al*., 2006). The disease is manifested in two forms subclinical and the clinical form. The subclinical form of the disease is subtle, and affects the welfare and productivity of birds in a manner that is difficult to notice until processing, when high numbers of carcasses and livers are condemned for low weight and

cholangiohepatitis (Stutz and Lawton, 1984., Lovland and Kaldhusdal, 1999). The clinical form of the disease is characterized by decreased feed intake, growth rate, and feed efficiency, with typical necrotic enteritis lesions in the intestine and up to 40% mortality rate (up to 40%; Tech Ross, 1999).

The effect of non antibiotic growth promotants such as probiotics/direct fed microbial products (Elwinger *et al*., 1992), prebiotics (Branton *et al*., 1997), blends of essential oil components (Mitsch *et al*., 2004), beta mannase feed enzyme (Jackson *et al*., 2003), on various intestinal pathogens has been investigated, and a partial level of protection has been reported. However, while most of these alternative therapies are somewhat beneficial, so far, none of them have demonstrated complete protection against gastrointestinal pathogens. Thus, it remains a priority for poultry scientists to identify an effective non-antibiotic supplement (or a suitable combination) that can completely replace the use of antibiotics for the control of CP and associated diseases.

Yeast extract, a non-antibiotic functional product essentially contains nucleotides and β-glucans which are immunostimulatory and possibly antimicrobial (Savage and Zakerwska, 1996; Santos *et al*., 2007). This property could be utilized for evaluating the effect of yeast products against intestinal pathogens of importance in broilers. In our previous study, NuPro® yeast extract supplement fed at 2% level for first 10 days of the broiler starter diet has demonstrated a potential to reduce intestinal CP levels. Hence we hypothesized that feeding NuPro® throughout the production cycle will significantly reduce CP levels in the intestine and associated clinical signs of necrotic enteritis in broiler chickens.

4.3 Materials and Methods

All the procedures used in this study were approved by the Auburn University Institutional Animal Care and Use Committee.

Experimental Design

Eight hundred day-old male broiler chicks (Cobb x Cobb) were obtained from a commercial hatchery, and transported to the Auburn University Poultry Research and Teaching Farm. Upon arrival at the farm, the chicks were weighed, and randomly assigned to one of eight dietary treatments (Table 4.3) in a completely randomized design. Treatment 1 (CX - control) consisted of chicks not challenged with CP and fed corn- SBM basal diet with no BMD or NuPro® added. Treatment 2 (CM) consisted of chicks not challenged with CP and fed corn-SBM basal diet into which BMD was added at 0.055 g/kg. Treatment 3 (SN) consisted of chicks not challenged with CP and fed corn-SBM basal diet supplemented with NuPro® at 2% level for the first 10 days of experiment as recommended by manufacturer (Alltech Inc., Nicholasville, KY). Treatment 4 (LN) consisted of chicks not challenged with CP and fed corn-SBM basal diet supplemented with NuPro® at 2% level throughout the experiment Treatment 5 (PX), treatment 6 (PM), treatment 7 (PSN), and treatment 8 (PLN) consisted of chicks that were challenged with 3 mL of CP inoculum (10^7 cfu/mL) on 3 consecutive days (days 14, 15, and 16) during the experiment. Diets fed to chicks in PX, PM, PSN and PLN were similar to diets fed to chicks in CX, CM, SN and LN treatments, respectively. Each treatment consisted of 4 replicate pens with 25 chicks per pen. Experimental diets (Table 4.1 and 4.2) were formulated to meet the recommendations of the National Research Council

(1994), and chicks were allowed *ad-libitum* access to feed and water throughout the experiment. Chicks were placed in floor pen house with fresh litter and maintained at $30^{o}C \pm 2^{o}C$ during the first week, after which temperature was reduced weekly by $2^{o}C$. Duration of experiment was 42 days.

Challenge Model and Preparation of CP Inoculum

The challenge model used in this experiment was a coccidia-free model designed as a modification of the models described by McReynolds *et al*. (2004) and Siragusa *et al*. (2008)**.** The three CP (type A) strains used for the challenge in this study were obtained from commercial poultry flocks having NE in different geographical locations (Texas, Virginia, and Georgia; McReynolds *et al*., 2004). To prepare the CP inoculum, each CP strain was streaked on 5% sheep's blood agar (Becton, Dickinson and Company, Sparks, MD) and incubated at 37°C for 24 hours inside anaerobic jars that utilized gas packs to generate anaerobic conditions (O_2 < 2% ; CO_2 = 9 – 13%; Mitsubishi Gas Chemical Company, Inc., Japan). Isolated white colonies with double zone of hemolysis obtained were inoculated into fluid thioglycolate broth (FTG, Oxoid Ltd, Hampshire, England) and incubated anaerobically at 37°C for 24 hours. A loop full of the FTG broth culture was streaked on tryptose sulfite cycloserine agar (TSC agar; Oxoid Ltd, Hampshire, England) and incubated anaerobically at 37°C for 24 hours. Black presumptive CP colonies were transferred into FTG broth and incubated anaerobically at 37°C for 18 hours. Thereafter, a cocktail of the three CP strains was prepared by mixing equal volumes of the FTG culture of each strain, resulting in a composite CP inoculum used for chick challenge. The concentration of CP cells in the inoculum was estimated

spectrophotometrically at 600 nm with the aid of a standard curve. Actual CP concentration in the inoculum was confirmed by plating on TSC plates, incubating the plates anaerobically at 37°C for 18 hours, and counting the numbers of black presumptive CP colonies. Confirmation of colonies as CP was performed using the Rapid ID 32 A test kit (BioMerieux, Durham, NC).

On days 14, 15 and 16 of experiment, a fresh composite CP inoculum was prepared daily, after which a round tipped animal feeding needle (15 G, 78 mm; Solomon Scientific, PA, USA) attached to a repeating syringe (Popper & Sons, Inc., New Hyde Park, NY) was used to orally deliver 3.5 mL of the inoculum into the crop of each chick in the PX, PM, PSN, and PLN treatments. Concentration of CP in the inoculum for each day was 8.6, 8.9, and 9.1 $\log_{10}$ cfu/mL on days 14, 15, and 16, respectively. The chicks in the control treatments (CX, CM, and CN) were orally gavaged daily (on days 14, 15, and 16) with 3.5 mL of freshly prepared sterile FTG broth.

Sampling for Intestinal Contents, Tissue Samples, and Lesion Scoring

Intestinal sampling to determine the concentration of CP in intestinal contents was done on days 13 (one day pre-challenge), 17 (one day post-challenge), 23 (7 days post-challenge) and 37 (21 day post-challenge) of experiment. On each day, two chicks were randomly taken from each pen (totaling 8 chicks per treatment) and euthanized by CO_2 asphyxiation. Thereafter, the intestine of each chick was aseptically excised, and a 15 cm cranial to Meckel's diverticulum section was removed and kept on ice for subsequent enumeration of CP on TSC media. On day 1 post challenge the duodenum was assessed for intestinal lesions using a scale described by (Prescott *et al*., 1978). Briefly, lesions

were scored on a 0 - 4 scale where 0 - no apparent lesions, 1 - thin friable small intestine, 2 - focal necrosis, ulceration or both, 3 - patchy necrosis, and 4 - severe extensive mucosal necrosis.

Determination of Intestinal CP Concentration

From each intestinal section collected for CP enumeration, approximately 5 g of intestinal content was weighed, transferred to 10 mL of anaerobic FTG broth, and stomached for 30 seconds (Stomacher 400 Circulator, Seward Limited, London, UK). One mL of the stomached digesta (1 mL) was transferred to 9 mL of anaerobic FTG broth, and serially diluted in FTG (ten-fold dilutions). The dilutions were plated on TSC agar, and incubated anaerobically at 37°C for 24 hours. The number of characteristic black colonies was then counted. Concentration of CP was finally expressed as $\log_{10}$ cfu/g intestinal content.

Performance Evaluation

Body weight, percent uniformity and feed conversion efficiency (FCE – calculated as feed-to-gain ratio) were recorded on d 21 and 42 of the experiment. Mortality was recorded daily.

Statistical Analysis

Using the General Linear Models (GLM) procedure of SAS (SAS Institute, 2004) intestinal CP concentration was transformed to $\log_{10}$ values and were subjected to one-way ANOVA along with, lesion scores and growth performance data (body weight,

percent uniformity and FCE). Significant differences among means were determined using the Duncan option of the GLM procedure as a post hoc test (Waller and Duncan, 1969; SAS Institute Inc., 2004). Mortality data after challenge was subjected to arcsine square root transformation, and subsequently analyzed by ANOVA, and Tukey test was applied for means separation (SAS Institute, 2004). Data are presented as means ± SEM. Statements of statistical significance were based upon $P < 0.05$.

4.4 Results and Discussions

Intestinal CP concentration and Lesion Scores

Intestinal CP concentrations are presented in Table 4.4. In all treatments, baseline CP concentration determined at one day pre-challenge (day 13 of experiment) showed no difference. This indicates that all chicks in the experiment probably had a similar concentration of intestinal CP at the beginning of the experiment, prior to CP challenge.

On day 1 post challenge, the uninfected treatments (CX, CM, SN, and LN) had a CP concentration that was significantly lower (P value < 0.005) than that of the infected treatments (PX, PM, PSN, and PLN) indicating a successful challenge. However there was no difference among the challenged treatment groups. This could be due to fact that the litter on floor pens serves as continues source of CP challenge due to the coprophagic activity of birds (Line *et al*., 1998) and the resultant CP colonization could not be effectively reduced by the effect of BMD or NuPro®. Even on day 7 post challenge, the CP concentration remained similar among the unchallenged treatments and the uninfected chicks still had a significantly lower (P value < 0.005) CP concentration when compared to the infected chicks, confirming that the CP infection established by day 1 post-

challenge was sustained till day 7 post-challenge. Results showed that supplementing NuPro® throughout the experiment reduced CP levels ($P < 0.05$; 1.51 $\log_{10}$ cfu/g reduction) compared PX treatment. On the other hand, the magnitude of intestinal CP reduced by PM (1.19 $\log_{10}$ cfu/g) and PSN (1.32 $\log_{10}$ cfu/g) was not significant. BMD is active against gram-positive bacteria, and is commonly included in commercial poultry diets for the control of CP-induced NE (Sims *et al*., 2004). Chicks fed NuPro® throughout the production cycle had similar performance to chicks fed BMD and better performance than the chicks kept on a control diet in reducing CP concentrations by the 21 day post challenge. The lesion scores of the chicks sampled day 1 PC is presented in figure 4.3. There was no difference in lesion scores between the different treatment groups. However the birds in PLN treatment had numerically lower lesion scores when compared to the birds in the other treatments. The exact mode of action of NuPro®-yeast extract in reducing intestinal CP-induced lesions is not clearly understood. *Saccharomyces cerevisiae* yeast increased cytokine production by macrophages (Adachi *et al*., 1994), and enhanced *in vitro* proliferative response of human lymphocytes (Darroch *et al*., 1994), NuPro®-yeast extract could also have stimulated the intestinal immune system to increase intestinal IgA production as found in dogs (Swanson *et al*., 2002), rats (Kudoh *et al*., 1999), and chickens (Gao *et al*., 2008) fed yeast supplements. IgA binds to antigens (perhaps such as CP α-toxin) and prevents them from passing through the mucosal membrane and establishing infection and lesions (Kulkarni *et al*., 2010). The immunomodulatory effect of yeast is attributed to its constituent nucleotides, mannan oligosachharides, and β glucans (Newman, 1994; Lowry *et al*., 2005; Chae *et al*., 2006;

Santos *et al.*, 2007). Hence addition of NuPro® throughout the production cycle had a positive effect in reducing CP colonization in the intestine of broiler chickens.

Necropsy Report

Necropsy reports of challenged birds from Thompson-Bishop-Sparks State Diagnostic Laboratory, Auburn, AL indicated dilated small intestine with fetid, dark red contents and variably mucoid to roughened mucosa. Also the birds showed liver infarctions. The findings were consistent with clinical NE caused by CP.

Growth Performance and Mortality

Effect of Nupro® on different performance parameters is presented in Table 4.5. The results were consistent with the previous battery trial showing no difference in body weight and FCR between the different treatments on both day 21 and 42 of trial. The FCR values by day 42 obtained in this study for the unchallenged birds in the yeast extract supplemented treatment (1.722) was comparable to those obtained by Morales-López *et al*. (2009,1.762) in which the birds were fed dietary beta glucans @ 145 mg /kg of feed. Feeding NuPro® throughout the experiment had no detrimental effect on performance parameters and was comparable to the effect of BMD throughout the study. Results of the effect of NuPro® on percent body weight uniformity during week 3 and 6 are presented in figure 4.1 and 4.2. The challenged birds had a trend of lower percent uniformity when compared to the unchallenged birds both during week3 and week6 (P value < 0.005). However during week 3 the birds in PLN treatment had a higher percent uniformity when compared to the other challenged treatments. A similar trend was also

noticed during week 6 where in the percent uniformity of the birds in PLN treatment was similar to the unchallenged treatments. Hence feeding NuPro® throughout the production cycle to the birds challenged with CP had shown a potential to enhance percent uniformity when compared to other challenged treatments. There is very less literature available on the effect of yeast and yeast products on the percent uniformity. However our findings were contrary to the results of Park *et al* (2005) where in the birds fed mannanoligosaccharide (a component of yeast cell) based diet did not show a difference in uniformity. The effect of NuPro® on post challenge mortality rates is presented in figure 4.4. We achieved 15% increase in mortality after the birds were challenged with CP which is consistent with the findings of McReynolds *et al* (2009). There was no significant difference in mortality rates between different treatments.

In conclusion, the effect of NuPro® on CP colonization, performance parameters such as body weight, FCR, percent uniformity, and mortality was comparable to effect of BMD throughout the study. Further investigation is required to understand the exact mechanism of action of NuPro® has against CP infection. NuPro® has significant potential to be used as an effective alternative to antibiotics for the control of NE in broiler chickens.

4.5. References

Adachi, Y., M. Okazaki, N. Ohno, and T. Yadomae. 1994. Enhancement of cytokine production by macrophages stimulated with (1-3)-*β*-D-glucan, grifolan (GRN), isolated from *Grifola frondrosa*. Biol. Phar. Bull. 17:1554–1560.

Branton, S. L., B. D. Lott, L. W. Deaton, W. R. Maslin, F. W. Austin, L. M. Pote, R. W. Keirs, M. A. Latour, and E. J. Day. 1997. The effect of added complex carbohydrates or added dietary fibers on necrotic enteritis lesions in broiler chicken. Poult. Sci. 76:24–28.

Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, and T. W. Hahn. 2006. Effects of supplementation of β-glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291–298.

Darroch, C. J., S. E. Christmas, and R. M. R. Barnes. 1994. In vitro human lymphocyte proliferative responses to a glycoprotein of the yeast *Saccharomyces cerevisiae*. Immunol. 81:247–252.

Douglas, M. W., M. Persia and C. M. Parsons 2003. Impact of galactose, lactose, and Grobiotic-B70 on growth performance and energy utilization when fed to broiler chicks. Poult. Sci. 82:1596–1601.

Eckles, C. H., and V. M. Williams. 1925. Yeast as a supplementary feed for lactating cows. J. Dairy Sci. 8:89–93

Elwinger. K. C., S. F. Berndtson, O. Fossum, B. Teglof, and B. Bagstrom. 1992. Factors affecting the incidence of necrotic enteritis, cecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. Acta. Vet. Scand 33:369-378.

Gao, J., H. J. Zhang, S. H. Yu, S. G. Wu, I. Yoon, J. Quigley, Y. P. Gao, and G. H. Qi. 2008. Effects of Yeast Culture in Broiler Diets on Performance and Immunomodulatory Functions. Poult. Sci. 87: 1377–1384.

Jackson, M. E., D. M. Anderson, H. Y. Hsiao, G. F. Mathis, and D.W. Fodge. 2003. Beneficial effect of beta mannase feed enzyme on performance of chicks challenged with *Eimeria* sp. and *Clostridium perfringens*. Avian Dis. 47:759-763.

Jensen, G. S., K. M. Patterson, and I. Yoon. 2008. Yeast culture has anti-inflammatory effects and specifically activates NK cells. Comp. Immunol. Microbiol. Infect. Dis 31: 487-500.

Jouany, J.P. 2001. A new look to at yeast culture as probiotics for ruminants. Feed Mix. 9:17–19.

Kaldhusdal, M., and Lovland. 2000. The economic importance of *Clostridium perfringens* is greater than anticipated. World's Poult. Sci. J. 16:50-51.

Kudoh, K., J. Shimizu, A. Ishiyama, M. Wada, T. Takita, Y. Kanke, and S. Innami. 1999. Secretion and excretion of immunoglobulin A to cecum and feces differ with type of indigestible saccharides. J. Nutr. Sci. Vitaminol. (Tokyo) 45:173–181.

Kulkarni, R.R., V.R. Parreira, Y.F. Jiang, and J.F. Prescott. 2010. A live oral recombinant *Salmonella enterica* serovar Typhimurium vaccine expressing *Clostridium perfringens* antigens confers protection against necrotic enteritis in broiler chickens. Clin. Vaccine Immunol. 17:205–214.

Line, E. J., J. S. Bailey, N. A. Cox, J. N. Stern, and T. Tompkins. 1998. Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. Poult. Sci. 77:405–410.

Lovland, A., and M. Kaldhusdal. 1999. Liver lesions seen at slaughter as an indicator of necrotic enteritis in broiler flocks. FEMS Immunol Med Microbiol 24:345–351.

Lowry, V. K., M. B. Farnell, P. J. Ferro, C. L. Swaggerty, A. Bahl, and M. H. Kogut. 2005. Purified β-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar *Enteritidis*. Int. J. Food Microbiol. 98:309–318.

Madrigal, S. A., S. E. Watkins, J. T. Skinner, M. H. Adams, A. L. Waldroup, and P. W. Waldroup, 1993. Effect of an active yeast culture on performance of broilers. Poultry Sci. 72 (Suppl. 1):87. (Abstr.)

McDevitt, R. M., J.D. Brooker, T. Acamovic, and N.H.C. Sparks. 2006. Necrotic enteritis; a continuing challenge for the poultry industry. World's Poult. Sci. J. 62: 221–247

McReynolds, J.,C. Waneck , J. Byrd , K. Genovese , S. Duke, and D. Nisbet. 2009. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. Poult. Sci. 88:2075-2080.

Mitsch, P. Z. E., K. Kohler, B. Gabler, C. Losa, and R. Zimpernik. 2004. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chicken Poult. Sci. 83:669-675.

Newman, K. E. 1994. Mannan-oligosaccharides: Natural polymers with significant impact on the gastrointestinal microflora and the immune system. Biotechnology in the feed industry. Pages 167–174 In T. P. Lyons and K. A. Jaques, ed. Proc. Alltech's 10th Annual Sym. Nottingham University Press, Loughborough, Leics, UK.

Parks, C. W., J. L. Grimes, and P. R. Ferket. 2005. Effects of virginiamycin and a mannanoligosaccharide-virginiamycin shuttle program on the growth and performance of large white female turkeys. Poult. Sci. 84:1967-1973.

Ross. Tech. 1999. Necrotic enteritis and associated conditions in broiler chicken. World Poult. 15:44–47.

Savage, T. F., and E. I. Zakrewska. 1996. The performance of male turkeys fed a starter diet containing mannan oligosaccharide supplementation (Biomos) from day-old to eight weeks of age. Pg 47–54. Proceedings Alltech's 12th Annual Symposium on the Biotechnological Feed industry.

Shin, Y. W., J. G. Kim, and K. Y. Whang. 2005a. Effect of supplemental mixed yeast culture and antibiotics on growth performance of weaned pigs. J. Anim. Sci. 83(Suppl. 1):34. (Abstr.)

Sims, M. D., K. A. Dawson, K. E. , P. Spring, and D. M. Hooge. 2004. Effects of dietary mannan oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. Poult. Sci. 83:1148-1154.

Solis de los Santos, F., A. M. Donoghue, M. B. Farnell, G. R. Huff, W. E. Huff, and D. J. Donoghue. 2007. Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune). Poult. Sci. 86:921–930.

Solomons I. A. 1978. Antibiotics in Animal Feeds—Human and Animal Safety Issues. J. Anim. Sci 46: 1360-1368

Stutz, M. W., and. G. C. Lawton. 1984. Effect of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens* and the ideal weight of broiler chicks. Poul. Sci. 63:2036–2042.

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, H. P. Healy, K. A. Dawson, N. R. Merchen, and G. C. Fahey. 2002. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. J. Nutr. 132:980–989.

Table 4.1. Composition of experiment 2 starter diet

Item	Diets[1]			
Ingredients (% of diet)	Corn-SBM Only	Corn-SBM with BMD	Corn-SBM with NuPro® for first 10 days of trial	Corn-SBM with NuPro® throughout trial
Corn	54.6	54.6	54.6	54.6
Soybean meal	36.8	36.8	36.8	36.8
Poultry fat	3.70	3.70	3.70	3.70
Dicalcium phosphate	1.74	1.74	1.74	1.74
Limestone	1.68	1.68	1.68	1.68
DL-Methionine	0.20	0.20	0.20	0.20
Salt	0.40	0.40	0.40	0.40
Trace minerals[2]	0.38	0.38	0.38	0.38
Vitamins[3]	0.75	0.75	0.75	0.75
Bacitracin (Antibiotic, g/kg)	-	0.05	-	-
NuPro® (First 10 days of Trial)	-	-	2.00	-
NuPro® (Throughout the Trial)	-	-	-	2.00
Calculated analysis				
CP (%)	23.00	23.00	23.00	23.00
ME kcal/kg	3200	3200	3200	3200
Calcium (%)	1.10	1.10	1.10	1.10
Available phosphorus (%)	0.13	0.13	0.13	0.13
Methionine (%)	0.54	0.54	0.54	0.54
Methionine + cystine (%)	0.90	0.90	0.90	0.90
Lysine (%)	1.25	1.25	1.25	1.25

[1]Diets include the corn soybean meal (SBM) basal, the corn-SBM basal diet supplemented with bacitracin methylene disalicylate (BMD) at 0.55 g/kg diet, corn-SBM basal supplemented with NuPro®-yeast extract at 2% level of the diet for first 10 days of the experiment, and corn-SBM basal supplemented with NuPro®-yeast extract at 2% level throughout the experiment.

[2]Auburn Chicken Trace Mineral Premix, Auburn University, Auburn, Alabama

[3]Auburn Chicken Vitamin Premix, Auburn University, Auburn, Alabama

Table 4.2. Composition of experiment 2 grower diet

Item	Diets[1]		
Ingredients (% of diet)	Corn-SBM Only	Corn-SBM with BMD	Corn-SBM with NuPro® throughout trial
Corn	65.11	65.11	65.11
Soybean meal	29.48	29.48	29.48
Poultry fat	1.54	1.54	1.54
Dicalcium phosphate	1.32	1.32	1.32
Limestone	1.43	1.43	1.43
DL-Methionine	0.07	0.07	0.07
Salt	0.30	0.30	0.30
Trace minerals[2]	0.25	0.25	0.25
Vitamins[3]	0.50	0.50	0.50
Bacitracin (Antibiotic, g/kg)	-	0.05	-
NuPro® (Throughout the Trial)	-	-	2.00
Calculated analysis			
CP (%)	20.00	20.00	20.00
ME kcal/kg	3200	3200	3200
Calcium (%)	0.90	0.90	0.90
Available phosphorus (%)	0.12	0.12	0.12
Methionine (%)	0.39	0.39	0.39
Methionine + cystine (%)	0.72	0.72	0.72
Lysine (%)	1.05	1.05	1.05

[1]Diets include the corn soybean meal (SBM) basal, the corn-SBM basal diet supplemented with bacitracin methylene disalicylate (BMD) at 0.55 g/kg diet, and the corn-SBM basal supplemented with NuPro®-yeast extract at 2% level of the diet

[2]Auburn Chicken Trace Mineral Premix, Auburn University, Auburn, Alabama

[3]Auburn Chicken Vitamin Premix, Auburn University, Auburn, Alabama

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Table 4.3. Description of experimental treatments (experiment2)

Treatment[1]	Challenge	Bacitracin	NuPro® (first 10 days of trial)	NuPro® (throughout the trial)
CX	-	-	-	-
CM	-	+	-	-
SN	-	-	+	-
LN	-	-	-	+
PX	+	-	-	-
PM	+	+	-	-
PSN	+	-	+	-
PLN	+	-	-	+

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (SN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatment 4 (LN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented throughout the experiment ;Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, 3, & 4 respectively.

Table 4.4. Effect of NuPro® supplementation on intestinal C. *perfringens* levels (experiment 2)

	Log cfu/g			
Treatment[1]	Baseline	Day 1PC[2]	Day 7PC	Day 21PC
CX	1.76	1.26^{b}	2.03^{c}	3.09^{ab}
CM	1.78	1.26^{b}	1.42^{c}	1.46^{c}
SN	1.26	1.26^{b}	1.97^{c}	1.67^{c}
LN	1.26	1.26^{b}	1.26^{c}	1.46^{c}
PX	2.41	4.19^{a}	4.37^{ab}	3.89^{a}
PM	1.26	2.84^{a}	3.45^{b}	2.70^{abc}
PSN	2.73	4.02^{a}	4.59^{a}	2.57^{abc}
PLN	1.26	2.85^{a}	3.93^{ab}	2.38^{bc}
SEM	0.318	0.472	0.326	0.426
P-value	0.1653	0.0001	0.0001	0.0023

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (SN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatment 4 (LN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented throughout the experiment ;Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, 3, & 4 respectively.

[2] PC – post challenge

Table 4.5. Effect of NuPro® supplementation on broiler performance (experiment 2)

Treatment[1]	Body weight(g)		Feed conversion ratio		
	wk3	wk6	wk3	wk6	Cum
CX	834	2803	1.349	1.794	1.642
CM	885	2959	1.359	1.777	1.631
SN	855	2996	1.366	1.736	1.614
LN	889	3094	1.345	1.722	1.594
PX	827	2794	1.373	1.992	1.716
PM	837	2756	1.356	1.856	1.639
PSN	802	2980	1.422	1.752	1.631
PLN	838	2991	1.373	1.751	1.615
SEM	18.273	87.367	0.018	0.071	0.0310
P-value	0.0557	0.1313	0.4412	0.2161	0.2800

[a-c] Means with different superscripts are significantly different.

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (SN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatment 4 (LN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented throughout the experiment ;Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, 3, & 4 respectively.

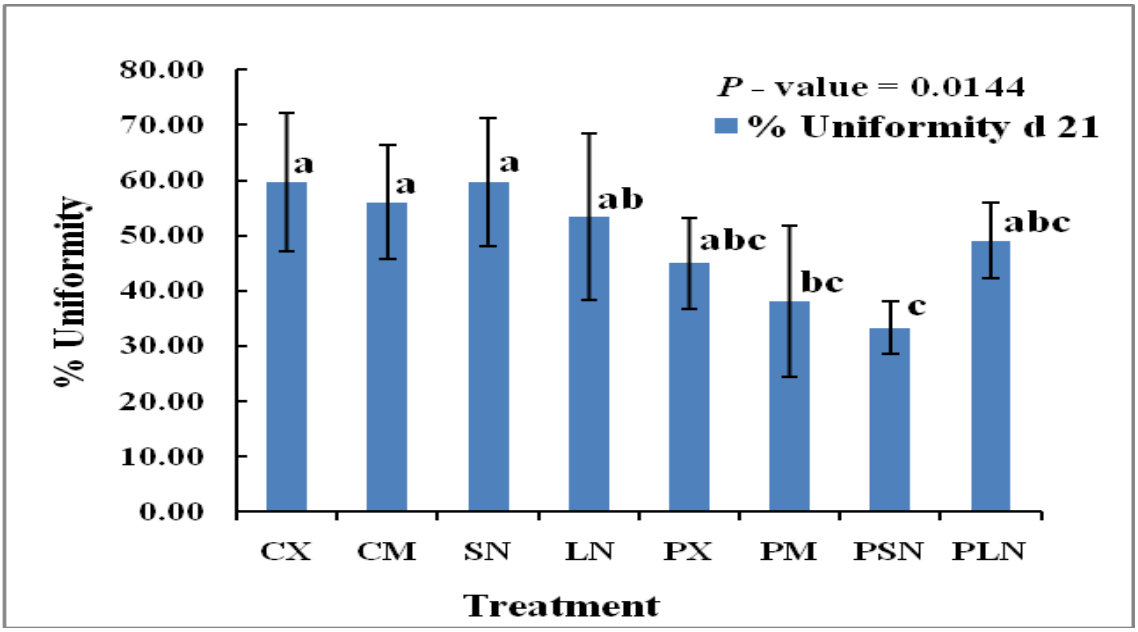
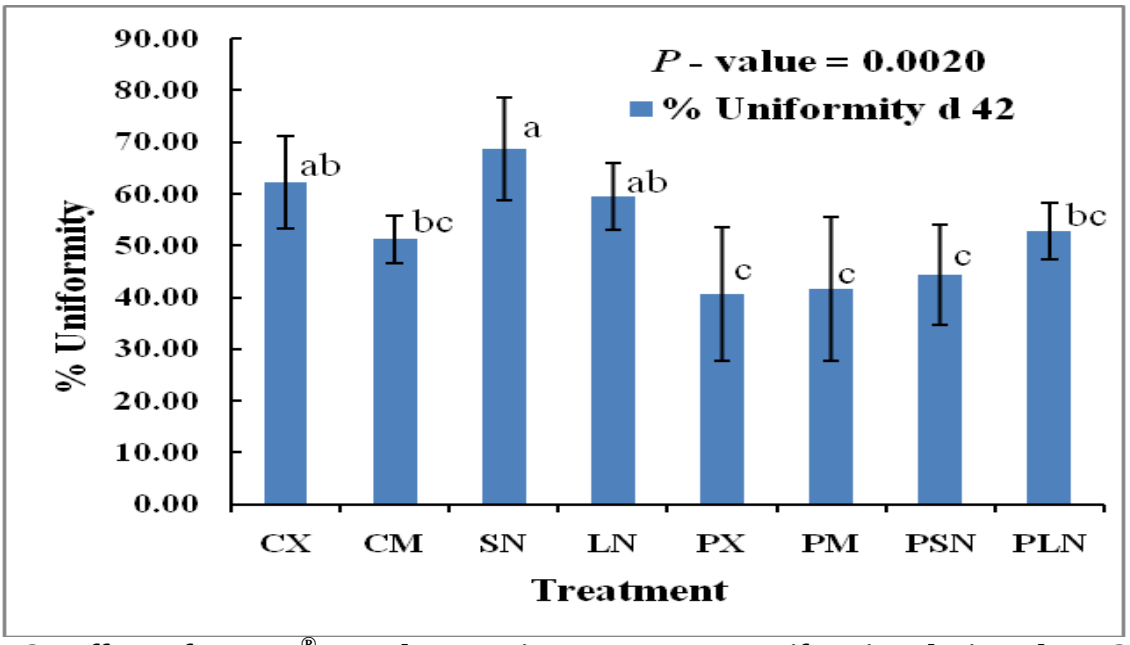


Figure 4.1. Effect of NuPro® supplementation on percent uniformity during day 21 (experiment 2).

Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (SN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatment 4 (LN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented throughout the experiment ;Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, 3, & 4 respectively.



4.2. Effect of NuPro® supplementation on percent uniformity during day 42 (experiment 2).

Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (SN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatment 4 (LN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented throughout the experiment ;Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, 3, & 4 respectively.

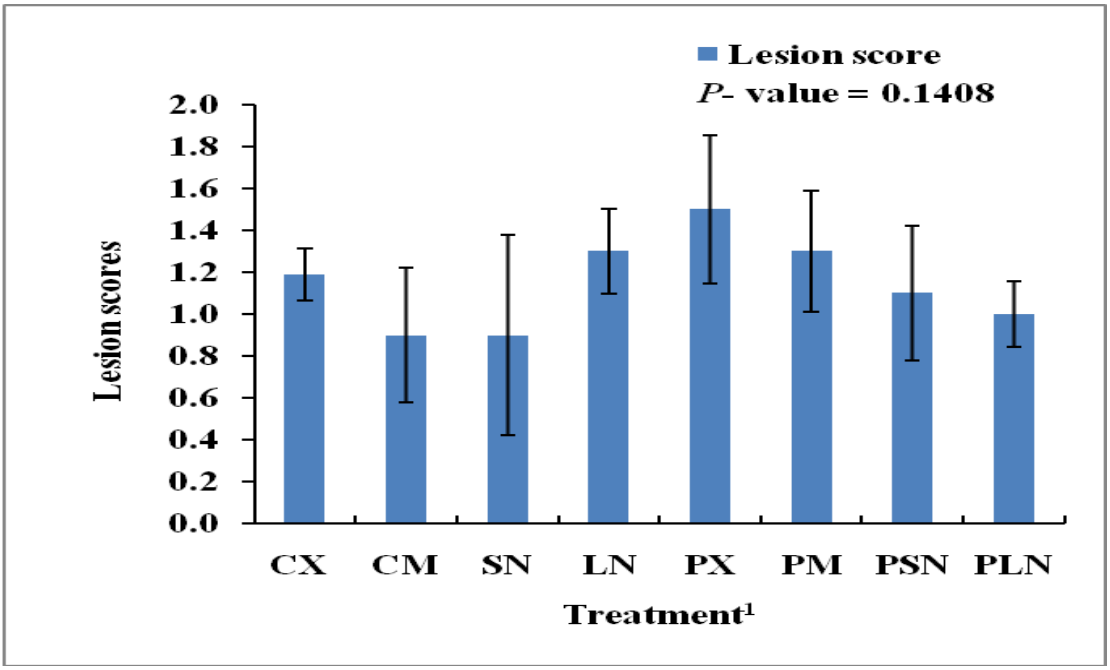


Figure 4.3. Effect of NuPro® supplementation on lesion scoring at day 1 post challenge (experiment 2).

[1]Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (SN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatment 4 (LN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented throughout the experiment ;Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, 3, & 4 respectively.

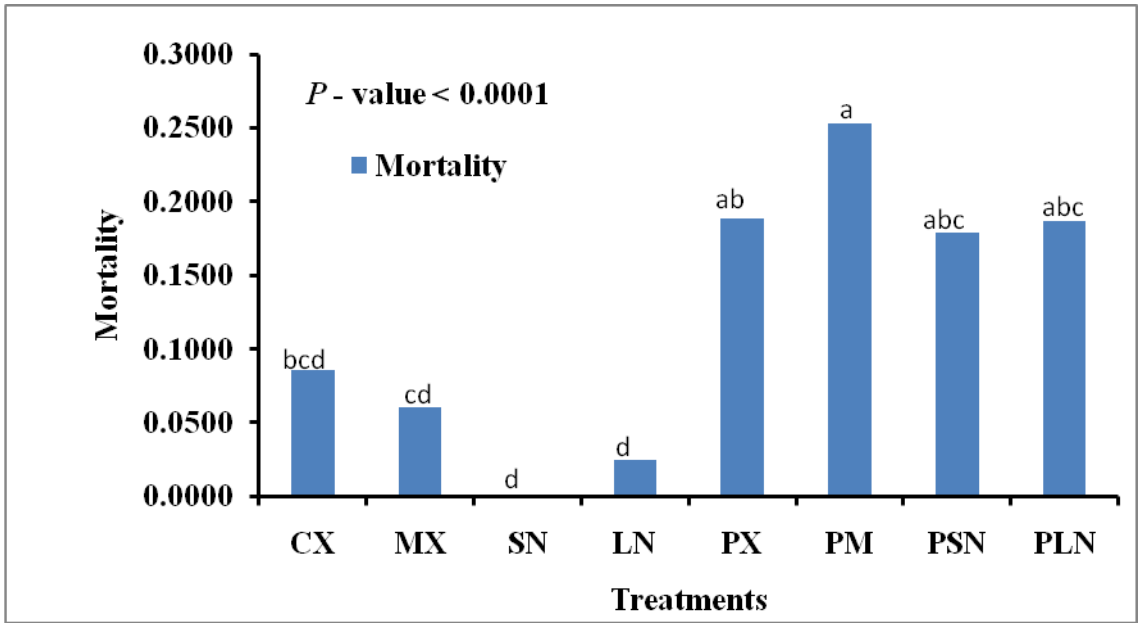


Figure 4.4. Effect of NuPro® supplementation on post challenge mortality rates. (experiment 2).

Treatment 1 (CX) consisted of unchallenged chicks fed with corn-soybean meal (SBM) basal only; Treatment 2 (CM) consisted of unchallenged chicks fed corn-SBM basal into which bacitracin methylene disalicylate antibiotic was supplemented at 0.055 g/kg diet; Treatment 3 (SN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented for the first 10 days of experiment; Treatment 4 (LN) consisted of unchallenged chicks fed corn-SBM basal into which NuPro®-yeast extract was supplemented throughout the experiment ;Treatments 5 (PX), 6 (PM), 7 (PSN) and 8 (PLN) consisted of chicks challenged with *C. perfringens* on three consecutive days (day 14, 15, and 16) and fed similar diets as chicks treatments 1, 2, 3, & 4 respectively.

V. CONCLUSIONS

Necrotic enteritis caused by *Clostidium perfringens* is an important enteric disease of poultry that causes economical losses to the poultry producers. Traditionally this disease was controlled by in-feed antibiotics. Due to the increased consumer demand for drug-free poultry, it remains a priority for poultry scientists to identify a suitable alternative to antibiotics. Dietary supplementation of yeast cultures have demonstrated some effect in modifying immune function by increasing the serum lysozyme levels, and activating natural killer cells and B lymphocytes in some animal species. In addition, it has been shown that yeast is effective in reducing *Salmonella* and *Campylobacter* colonization in broiler chicks subjected to transport stress. In another study, yeat extract supplemented at the rate of 1 g/kg of feed in turkey poults had significantly enhanced lamina propria thickness, crypt depth, and mucin-producing goblet cells in the ileum, and to some degree in the jejunum and duodenum, compared to the control group. Since *C. perfringens* causes extensive damage to the gastrointestinal tract yeast products may serve as suitable non-antibiotic alternatives for controlling intestinal CP colonization and NE.

Two experiments were conducted to evaluate the efficacy of NuPro®-yeast extract supplementation in reducing clinical signs associated with CP and NE. To conduct this investigation we experimentally induced CP infection and NE in the intestine of broiler chickens. Results of both the experiments showed that supplementing NuPro®-yeast

extract into the diet of broiler chicks is effective in reducing intestinal CP and clinical signs of NE without causing any adverse effect on chick growth performance. The first experiment was a 25 day battery trial in which subclinical NE was experimentally induced. Results showed that supplementing NuPro® for the first 10 days of the experiment decreased intestinal CP levels by 1.73 $\log_{10}$ cfu/g and 0.68 $\log_{10}$ cfu/g at 1- and 7-days post-challenge periods. The second experiment was a 42 day floor pen trial in which clinical NE was experimentally induced. Results showed that supplementing NuPro® throughout the experiment reduced CP levels ($P < 0.05$; 1.51 $\log_{10}$ cfu/g reduction) compared to the positive control treatment. On the other hand, the magnitude of intestinal CP reduced by BMD (1.19 $\log_{10}$ cfu/g) and NuPro® fed for 10 days (1.32 $\log_{10}$ cfu/g) was not significant.

In conclusion, the effect of NuPro® on CP colonization, performance parameters such as body weight, feed conversion, percent uniformity, and mortality was comparable to effect of BMD throughout the study. Although further investigation is required to understand the exact mechanism of action of NuPro® against CP infection, NuPro® has significant potential to be used as an effective alternative to antibiotics for the control of NE in broiler chickens.