STUDY OF THE YEAST CELL WALL COMPONENTS, MANNAN OLIGOSACCHARIDE AND BETA GLUCAN, TO DETERMINE THEIR INDIVIDUAL AND SYNERGISTIC INFLUENCE ON BROILER PERFORMANCE UNDER PATHOLOGIC STRESS

A Dissertation

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ABSTRACT

Different supplementations or strategies have been proposed as alternatives to the use of antibiotics at sub-therapeutic levels in chickens. Mannan oligosaccharides (MOS) and β-glucans have been reported to beneficially influence broiler performance and health. However, different purifications levels have been employed to investigate the efficacy of those oligosaccharides, and thus conflicts have been noticed with respect to their influences.

A series of studies were conducted to investigate the influence of purification, source, and individual components of yeast cell wall (YCW) on broiler performance subjected to pathogenic challenge. The challenge model included administration of 10x the recommended level of infectious bursal disease vaccine via the ocular route to all birds on day 10, in order to immunocompromise them. On days 16 and 17, birds in all treatments except non-challenged control received 3 mL of 10^7 cfu of *Clostridium perfringens* (Cp). Broiler performance was evaluated on days 10, 16, and 21. In study 1, tissue samples were collected from the jejunum and duodenum regions for analysis with a chicken-specific peptide arrays technique to study the influence of YCW supplementation on the immune and metabolic pathways.

The Cp challenge significantly impacted broiler performance in all studies. The purification level of YCW preparations had different levels of influence on performance as well as metabolic pathways. The addition of purified YCW to the starter broiler diets improved performance and influenced the immune and metabolic pathways in the gut.

In the second study, two experiments were conducted with different levels of highly purified β-glucan from algae (*Euglena gracilis*) or highly purified MOS from *Saccharomyces cerevisiae*. Addition of no more than 30 ppm of algae β-glucan was sufficient to improve broiler performance; however, no significant differences were observed in performance with any of the MOS levels.

The third study evaluated the influence of mannoproteins or β-glucan preparations purified at 50-55%, derived from the same YCW product of *Saccharomyces cerevisiae* individually or in combination. The performance of starter broilers was improved when a combination of mannoprotein and β-glucan was added to the diet.

The last study evaluated the influence of two YCW products prepared from two different strains of *Saccharomyces cerevisiae* on full-term broiler performance for birds reared on reused floor litter. The products were supplemented at either 250 ppm continuously or 500, 250, and 125 ppm in the starter, grower, and finisher phases, respectively. Broiler performance positively responded to YCW products and both feeding strategies. Overall, purification and source of β-glucan and MOS have significant impacts on broiler response, and those components have synergetic influence when combined.

DEDICATION

This work is dedicated to my parents, Mr. Malik Hashim and Mrs. Fadheelah Ibrahim.

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NOMENCLATURE

AGP	Antibiotic Growth Promoter
AMPK	5'-Adenosine Monophosphate-activated Protein Kinase
BG	β-glucan
Cp	*Clostridium perfringens*
mTOR	mammalian target of rapamycin
MOS	Mannan oligosaccharides
NE	Necrotic Enteritis
NK cells	Natural Killer cells
YCW	Yeast Cell Wall

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Yeast cell wall

The rigidity of the cell wall maintains the shape of the yeast and provides protection to prevent cell rupture due to internal pressure. Yeast cell wall (YCW) has an anionic surface and its fibrous composition depends on β-1,3- and β-1,4-linked polysaccharides that form helical (β-1,3-glucan) or ribbon-like (chitin and cellulose) structures (Lipke and Ovalle, 1998). The cell wall of *Saccharomyces cerevisiae* comprises approximately 15-30 percent of the dry weight of vegetative yeast (Orlean, 1997). A quantitative chemical analysis revealed that the chemical composition of yeast cell wall consists of 3% ash, 13% protein, 8.5% lipid (mostly neutral fat), and two major polysaccharides: glucan (29%) and mannan (31%); the latter is associated with protein present in the wall to form mannoprotein complexes (Northcote and Horne, 1952). The mannan is linked to the protein by N-links, and this complex is attached covalently to a short chain β-1,6-glucan by a glycosylphosphatidylinositol anchor protein (Kollár et al., 1997). Another component of YCW is chitin, which is present in a very negligible amount of about 1-2% and mostly occurs in the division septum (Orlean, 1997). The composition and/or percent makeup of YCW components are not always constant. The ratio of the mannose polymers (mannoproteins) to glucose polymers (β-1,3-glucan and

β-1,6-glucan) and chitin in the cell wall seems to be somewhat dependent on the growth medium or fermentation conditions (Aguilar-Uscanga and François, 2003).

Gastrointestinal tract

The gastrointestinal tract has important biological functions that include digestion of food, secretion of digestive elements, absorption of nutrients, and excretion of waste materials. The mucosa of the gastrointestinal tract is the largest mucosal surface and is considered a pivotal location for innate and adaptive immune responses because of its direct contact with nutritional antigens and various microorganisms (Turner, 2009). The mucosal barrier of the GI tract is composed of the extracellular components mucin and an unstirred layer, as well as cellular components. Mucins are heavily glycosylated proteins that are secreted by specialized mucosal epithelial cells such as goblet cells and play a vital role in preventing direct contact between the epithelial layer and microbes (Turner, 2009). The epithelial cell plasma membrane also serves as a mucosal barrier, thus any direct damage to the epithelial cell causes interruption to the barrier function. The apical junction complex, which consists of adherens and tight junctions, regulates the function of the paracellular pathway in healthy epithelial cells and a ring of myosin and actin (Turner, 2009) reinforces those junctions. Tight junctions are complexes of multi-proteins consisting of transmembrane proteins, peripheral membrane proteins, and regulatory molecules such as kinases (Turner, 2009).

The digestive tract is occupied by tremendous amounts of microbiota that can produce beneficial or harmful compounds to the host by fermentation processes. This microflora can be altered due to environmental or nutritional changes, and such

alterations can have physiological or systemic influences on the host. An example of a microbe that exists in the gastrointestinal tract is *Clostridium perfringens*, which is a gram positive, spore forming, anaerobic bacilli, and exotoxin and enterotoxin producing bacteria. This bacterium is opportunistic and can proliferate rapidly under conditions that may not be favorable to the host. It has been linked to clinical or subclinical necrotic enteritis and leads to adverse effects on host health and performance. This pathogen causes significant economic loss to the U.S. poultry industry. In addition, cases of foodborne illness in humans have been linked to this pathogen. The use of antibiotics at sub-therapeutic levels as growth promoters has helped in controlling the incidence of necrotic enteritis in poultry. However, antibiotic-free poultry products and consumer demands toward banning the prophylactic use of antibiotics encourage development of antibiotic-free feeds that can help control pathogenic organisms such as *Clostridium perfringens*.

Yeast cell wall as an antibiotic alternative

Gibson and Roberfroid (1995) introduced the concept of prebiotics as nondigestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, resulting in improved host health. Many compounds have been recognized as prebiotics. Studies have demonstrated that YCW is capable of reducing pathogenic colonization and altering microbial structure in the gastrointestinal tract (Calveyra et al., 2012; Corrigan et al., 2011; Spring et al., 2000; Thanissery et al., 2010). Mannan oligosaccharides (MOS), one of the main components of YCW, are able to bind to some pathogenic bacteria and thus

remove those bacteria from the intestine as well as enhance the immune status of the host (Spring et al., 2000). These oligosaccharides have the ability to inhibit the attachment of bacteria with Type-1 fimbriae, such as strains of *Salmonella* and *E. coli*, to the intestinal mucosa (Spring et al., 2000). In addition, YCW has the ability to modify the microbial community in the colon and increase the population of some beneficial bacteria such as *Lactobacillus* (Hashim et al., 2013). Glucan has also shown some prebiotic activity on some probiotic bacteria as shown in a study by Russo et al. (2012), who reported that β-D-glucan positively influenced the growth of *Lactobacillus* strains in vitro.

Kinomics

The influence of intestinal microbial system alteration on host performance has been studied for many years. The use of YCW or its components as alternative growth promotors has proven to alter gut microbiota and enhance animal growth. Studies have demonstrated that 5'-adenosine monophosphate-activated protein kinase (AMPK) enzyme activity is influenced when there is change in the intestinal microflora via an unclear mechanism (Arsenault et al., 2013). Kinases catalyze the hydrolysis of phosphate groups from ATP; kinomics is the study of these kinase enzyme groups. Several techniques are being used to investigate cellular signaling through phosphorylation. The peptide arrays technique is one such technique. As its name implies, this procedure uses peptides that represent the target site of kinase enzymes, and an array surface is used to synthesize and print these peptides. Then, a mixture of purified kinases or a cellular lysate that represent active protein kinases is applied to the

array and recognizes their corresponding target sequences. Phosphorylation due to added adenosine triphosphate (ATP) is visualized via several methods such as radioactivity, antibody phosphorylation, or phosphor-specific stains using fluorescent phosphorylation sensor dye (Arsenault and Kogut, 2012).

Some enzymatic proteins may not catalyze their reaction and influence their substrates because of no activating post-translating modification, or due to protein sequestering within an organelle (Arsenault and Kogut, 2012). Most signal transductions in eukaryotic cells are mediated by protein kinases, which also regulate several cell processes including but not limited to metabolism, apoptosis, and cell differentiation. In addition, protein phosphorylation is pivotal to intercellular communication during development or functioning of the immune system (Manning et al., 2002). The peptide array technique focuses on active kinase enzyme sequence rather than complete protein sequence because full-length protein is not always stable on an array format (Arsenault and Kogut, 2012). Phosphorylated proteins commonly have multiple phosphorylation sites and thus have different protein behaviors, such as one site activating enzyme catalyst activity and another site deactivating this activity (Arsenault and Kogut, 2012; Gottipati et al., 2008; Smith et al., 2009). Therefore, important information can be obtained from these phosphorylation activities, and they can be separated from each other by printing these specific phosphorylation sites as separated peptides on the array (Arsenault and Kogut, 2012). Since phosphorylation controls the cellular signaling pathways that regulate metabolism, chicken-specific kinomic techniques can be

employed to understand any change in the metabolic process, such as activation or deactivation (Diks et al., 2004).

To the best of our knowledge, there is no comprehensive study that investigates the differences between the components of YCW on broiler performance and reveals their individual mechanisms of action. In order to better understand the influence and mechanism of YCW components on broiler performance, the glucan and mannan components will be investigated in a series of experiments. First, the influence of YCW purification on broiler performance will be evaluated. In addition, a chicken-specific peptide arrays technique will be employed to evaluate the differences in immune and metabolic pathways that can be observed due to different purification level or component of YCW. Then, separate experiments will be conducted to assess different levels of algae β-glucan, and yeast mannan oligosaccharides (MOS) and compare them to intact YCW. Third, purified yeast MOS and glucan subcomponents will be compared either individually or in combination to YCW. Finally, two different supplementation strategies of primary or brewer's YCW strains will be investigated in broiler floor study.

Literature Review

Saccharomyces cerevisiae

Saccharomyces cerevisiae is a species of yeast and a normal habitant of soil, and it can be found associated with fruits and vegetables. It belongs to the fungi kingdom because its cell wall contains chitin. The shape of this yeast is ellipsoidal ovoid to round and has a diameter of approximately 5-10 micrometers. It reproduces through asexual

reproduction known as “budding” and takes about 1.5 hours to divide. It was used in beverage fermentation about 9000 years ago by the Chinese and later used by the Persians in wine, as well as the ancient Egyptians in bread baking (Anonymous, 2015; Legras et al., 2007). In 1856, Luis Pasteur found that this yeast is a facultative anaerobic microorganism and has a crucial role in wine making and bread baking processes (Anonymous, 2015).

Although no toxins have been isolated from *Saccharomyces cerevisiae* that can be harmful to humans or animals, this yeast can produce anti-yeast toxins called killer toxins which are classified as proteins or glycoproteins. Phospholipase A and lysophospholipase improve the ability of the yeast to attach to the cell wall surface and cause colonization to start the yeast infection process. Thus, these enzymes are virulence factors that are used to measure the degree of virulence of the yeasts (EPA, 1997). In order to consider yeast as nonpathogenic, it should have low phospholipase activity. *Saccharomyces cerevisiae* has the lowest enzyme activity among all other yeasts that have been tested and thus is considered as a nonpathogenic yeast (Barrett-Bee et al., 1985). Ability of yeast to compromise the immune system of the host is another factor that is used to determine the virulence of the yeast. Colonization of *Saccharomyces cerevisiae* was greater in animals exposed to this yeast and treated with cortisone as an immunosuppressant factor compared with animals exposed to yeast only. However, both groups of animals had no illness effects, which suggests that this yeast is nonpathogenic (Holzschu et al., 1979).

Legras et al. (2007) isolated 651 strains of *Saccharomyces cerevisiae* that were collected from 56 different regions around the world. These strains were grouped into three main categories according to their substrates that included wine, bread, and beer, and some strains were isolated from fermented milk and cheese. The differentiation between these strains is a consequence of technological changes throughout human history and genetic drift factors.

The cell wall of *Saccharomyces cerevisiae* provides physical and osmotic protection and maintains the shape of the yeast (Smith et al., 2000). The wall consists of two layers: an outer layer which is electron-dense and consists mainly of mannoproteins, and an inner layer which contributes approximately 50-60% of the dry weight of the wall, is electron-transparent, provides most of the mechanical support, and consists of β-1, 3-glucan and chitin (Klis et al., 2002b). The cell wall proteins are connected by covalent links to the β-1, 3-glucan and chitin either directly or indirectly via β-1, 6-glucan (Klis et al., 2002b).

One of the disadvantages of employing the yeast in the diet is the low digestibility of the cell wall, which can be improved via enzymatic hydrolysis and decreasing the DNA and RNA volume (Tukmechi and Bandboni, 2014). Production of yeast extract is conducted by removing the cell wall of the yeast completely; the final product has better digestibility, with higher protein content than either the yeast itself or hydrolyzed yeast (Tukmechi and Bandboni, 2014). The extract of *Saccharomyces cerevisiae* has been investigated to study its effects on performance and health of both humans and animals. One of those studies examined the influence of the yeast extract

added to cosmetic formulation with or without vitamins A, E, and C on skin moisture and skin microrelief after 3 hours, 15, and 30 days of application (Gaspar et al., 2008). This study found that the yeast extract improved skin brightness, and this treatment or its combination with the vitamins prolonged the skin microrelief effect.

Another study was conducted to evaluate the effect of supplementation of baker's yeast extract and/or hydrolyzed yeast powder on hematological and immunological parameters of rainbow trout (Tukmechi and Bandboni, 2014). This study concluded that a combination of yeast extract and hydrolyzed yeast powder, each at 0.5%, increased the total leukocyte counts, neutrophils, lymphocytes, and monocytes percentages. In addition, the combination treatment had significantly higher lysosome activity and total antibody levels than all other treatments on days 30 and 60 of the study. While yeast extract alone produced a higher antibody level than hydrolyzed powder or control treatments on day 30 and only higher than control on day 60, it had higher lysosome activity than the control only on day 30 and both treatments alone were higher than control on day 60. The findings of this study suggested that a combination of yeast extract and hydrolyzed yeast powder improved the health of the rainbow trout.

In poultry, much research has been conducted to assess the administration of yeast extract or YCW on performance and health of different poultry species. The encouraging results of these studies increased attention paid to the prospect of using these supplements to replace antibiotic growth promoters. One of these studies examined the influence of adding 0.1% of a YCW derivative product that contains 26% β-glucan and 15% MOS on broiler breeders fed a diet contaminated with 100 ppb aflatoxin

(Matur et al., 2011). The study found that yeast wall extract effectively ameliorated the negative effects of aflatoxin on heterophil and lymphocyte percentages. Although aflatoxin did not negatively affect the phagocytic activity percent, addition of yeast extract positively improved this innate immune system variable. Another study was conducted to evaluate the effect of yeast extract containing standardized portions of β-glucan and MOS on body weight, hematologic parameters, and immunologic variables of Hybrid Converter turkey poults challenged with *E. coli* and transportation stress (Huff et al., 2010). It was reported that adding yeast extract at 1000 g/ton significantly increased body weight of the control group, but not the stressed treatment. The bacterial and transportation stresses decreased the total leukocytes count and percentage of lymphocytes and increased the heterophils: lymphocytes ratio and heterophils percentage, but the changes were not observed in birds fed the supplemented diet. Hemoglobin concentration and hematocrit were significantly higher in stressed poults fed on diet supplemented with 1000 g/ton than control stressed poults. This finding suggested that YCW extract can be used as an immunomodulatory and can be considered a developing alternative to antibiotics in turkey. Feeding male broilers a basal diet vs diet supplemented with either yeast extract or bacitracin methylene disalicylate with or without *Clostridium perfringens* challenge was also investigated (Thanissery et al., 2010). This research trial reported that on the day after challenge, the concentration of *Clostridium perfringens* in the small intestine was significantly lower in birds fed the diet with antibiotic (2.09 $\log_{10}$ cfu/g) than the control challenged birds (4.71 $\log_{10}$ cfu/g), and the challenged birds fed a diet supplemented with yeast extract had a pathogenic

concentration (2.98 $\log_{10}$ cfu/g) comparable to the antibiotic treatment. Yeast extract was fed to the birds for the first ten days prior to challenge at 2% of the diet instead of lower concentration throughout the study, which could be the reason for not having a significant reduction in *Clostridium* concentration compared to control challenged group. YCW extract was also investigated at three different levels throughout the study (800, 400, and 200 mg/kg of diet for 10, 14, and 11 days) to examine its efficacy compared to antibiotic zinc bacitracin (100 mg/kg of diet10 days and 50 mg/kg of diet for 25 days) or antibacterial coccidiostat ionophore salinomycin (60 mg/kg for the entire time of the study) on broilers challenged with attenuated vaccine strain sporulated oocysts of *Eimeria maxima, E. acervulina*, and *E. brunetti* on day 9 and then challenged with *Clostridium perfringens* on day 14 (M'Sadeq et al., 2015). This study reported no significant differences in feed intake between challenged treatments that were supplemented with YCW extract, salinomycin, or zinc bacitracin on days 24 or 35. Although weight gain was significantly higher in salinomycin treatment than both zinc bacitracin and yeast extract treatments (which both exhibited the same weight gain results), all challenged supplemented treatments were significantly higher than challenged control on day 24, diminishing the difference in weight gain among the supplemented treatments. Intestinal integrity was positively influenced with yeast cell extract via increasing villus height, decreasing crypt depth, and increasing villus height: crypt depth ratio in the jejunum, which can explain the improvement in performance in those birds. The effect of live heat-stable yeast culture or different sources of its derivatives such as baker's or brewer's yeast was investigated in series of trials on

performance of starter broilers that were subjected to immune stress by infectious bursa disease vaccine followed by *Clostridium perfringens* challenge (Fowler et al., 2015b). This group of studies revealed that the origin of YCW product has no effect on broiler performance; however, blending baker's and brewer's YCW derivatives at 50% each with an inclusion rate of 135 ppm improved body weight significantly more than control and both yeast derivatives alone. In addition, the dose of YCW affected body weight in a quadratic response, and a 250 ppm inclusion rate had a better response than 125 or 500 ppm.

Another study was conducted to assess the amelioration effect of adding heat-stable live yeast culture or YCW extract products to the diet of full-term broilers fed on a corn/soy diet or a variable ingredients diets (Fowler et al., 2015a). This study indicated that feeding broilers a diet composed of variable ingredients negatively influenced body weight and weight gain, and adding YCW or live yeast culture mitigated this impact. Performance of broilers fed diet supplemented with live yeast was as good as the performance of control group that fed on a corn/soybean meal based diet, especially during the starter phase.

Mycotoxins are secondary metabolites of several fungi genera and adversely affect human and animal health and performance. These mycotoxins can be found in several agricultural crops such corn, soybean, and peanut. YCW adsorbs mycotoxins and thus diminishes their impacts. Adsorption capabilities vary depending on the dose of YCW and the type and concentration of mycotoxin (Zekovic et al., 2005).

It can be concluded from these studies that supplementing diet with YCW and/or yeast extract has positive effects on birds' performance, immune response, and intestinal integrity. These influences are more perceptible when birds are under stress such as environmental, nutritional, or pathogenic challenges.

Glucans

Glucans are polysaccharides that exist as natural components in plants, cereals, bacteria, algae, fungi, and yeast. The source of beta glucans influences their structures by determining how their β-D-glucose molecules are linked by glycosidic bonds (Jacob and Pescatore, 2014). Most beta glucans of cereal grains such as barley are unbranched with 1, 4-β bonds, while bacterial beta glucans are unbranched with 1, 3-β-links. The glucans of YCW are composed of long chain β 1, 3-glucan and short chain β 1, 6-glucan. The β 1,3-glucan chains have hollow helix structures consisting of approximately 1500 glucose monomers in partially hydrolyzed glucan chains that may vary depending on the extracted acid, growth stage and carbon source (Klis et al., 2002a). These helix structures make YCW elastic. In addition , β 1,6-glucose residues contribute about 3-4% of the mature moderate branched form of β 1,3-glucan (Manners et al., 1973). The literature has reported different percentages of these glucan parts in the mass of YCW. β 1, 3-glucan comprises about 40-55% and β 1, 6-glucan comprises 5-10% of the dry weight of the wall, and these percentages vary with the different growth conditions of the yeast (Klis et al., 2002b; Lipke and Ovalle, 1998).

The biological responses to beta glucan depend on several factors including extraction procedure, solubility, molecular weight, branching, and source of the glucans.

Several studies have investigated biological influence of glucans on the host. However, most of those studies have not used pure glucan; other polysaccharides have been associated with their glucan. This has been especially true for livestock animal trials. However, *in vitro* or human model trials have been more advanced in this aspect. β 1,3-glucan is in the biological response modifiers drug class because it can stimulate the host's immune system and thus modifies the biological response of the host (Zekovic et al., 2005). Although macrophages are the part of the immune system that is most influenced by β 1,3-glucan, other pathways are also activated such as natural killer cells (NK), T-cells, the reticuloendothelial system, and antibodies titration; thus, β 1,3-glucan is classified as a non-specific immunomodulator (Zekovic et al., 2005).

The immuno-stimulatory mechanism of β 1,3-glucan in patients infected with the severe fungal disease paracoccidioidomycosis was investigated to evaluate the influence of beta glucan when those patients are treated with antifungal medicine that has an immunosuppression effect (Meira et al., 1996). Glucan significantly increased tumor necrosis factor (TNF); cytokines released from macrophages acted as an immunologic mediator that protected the host by stimulating the macrophages via Toll-Like Receptors 2 (TLR2) mediation. In addition to TNF, the glucan activates macrophages by increasing their size and number, stimulating lysozyme secretion, and increasing antigen phagocytosis (Zekovic et al., 2005). Moreover, activation response has been observed by beta glucan in other immune cells such as monocytes, neutrophils, eosinophils, and non-immune cells such as fibroblasts, alveolar epithelial cells, and endothelial cells (Brown and Gordon, 2003).

Beside the immune activity, glucan has antitumor activity that has been investigated and reported to have a similar mechanism of action. One study indicated that β 1,3-glucan has both proliferated influence on normal bone marrow and spleen cells and direct cytostatic influence on tumor cells when the latter was co-incubated with glucan *in vitro* (Williams et al., 1985). Another study demonstrated that soluble (40% soluble glucan and 60% glucose) or particulate β 1,3-glucan of *Saccharomyces cerevisiae* can inhibit growth significantly in melanoma B16 and syngeneic anaplastic carcinoma as well as improve the longevity and decrease mortality rate of mice with subcutaneous tumor implants (Di Luzio et al., 1979). There are two receptors that determine the biological response to beta glucan. One of the receptors is inactivated complement component 3 (iC3b), also known as CR3, which is mainly present on neutrophils, monocytes, NK cells, and to lesser extent on macrophages. The second receptor is Dectin-1, which is more expressed on macrophages than neutrophils, and is not expressed on NK cells (Chan et al., 2009). The pharmacodynamics of fluorescein-labeled soluble barley and particulate yeast β 1,3-glucan was investigated by an *in vivo* study in mice to explain its antitumor activity (Hong et al., 2004). The orally administered glucan was taken up and transported by gastrointestinal macrophages to bone marrow (primary lymphoid organ) and secondary lymphoid organs (spleen and lymph nodes). Then, the macrophages in the bone marrow broke down the large beta glucan particles into smaller soluble fragments, and the latter bound to the CR3 receptors of the granulocytes to be shuttled to the site of inflammation where they killed tumor cells that were iC3b-coated.

The phagocytes such as macrophages, neutrophils (heterophils in avian species), and dendritic cells express special receptors are known as pattern recognition receptors (PRRs) that are able to recognize simple molecules and regular patterns of molecular structure defined as pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) (Murphy et al., 2008). The immunomodulatory activities of β 1,3-glucan explained the antimicrobial characteristic that is proposed with fungi- and yeast-origin beta glucans due to their function as MAMPs (Jacob and Pescatore, 2014). The phagocytic and bactericidal functions against *Salmonella enteritidis*, as well as the oxidative burst of heterophils isolated from chicks, were improved significantly when the chicks were fed a diet supplemented with purified beta glucan (Lowry et al., 2005). Another study was conducted to evaluate the modulating influence of yeast beta glucans on performance and immune response of broilers (Zhang et al., 2008). Five levels (25, 50, 75, 100, and 125 ppm) of 91.5% β 1,3 / 1,6-glucans were fed to male broilers and body weight, feed intake, and blood samples were taken on days 21 and 42. The results demonstrated a quadratic effect of glucan level on performance and immunological parameters. Feeding 50 or 75 mg/kg of glucans significantly increased body weight and decreased feed-to-weight gain ratio on days 21 and 42. Interleukin-1 and 2, interferon γ, and TNF-α were significantly higher in 50 mg/kg glucans level than control. Serum IgG, plasma globulin, and intestinal secretory IgA were significantly higher at 50 and 75 mg/kg glucans. These finding suggested that 50 mg/kg of diet maximize both performance and humoral immune responses in broilers. Fungal beta glucans have similar structure to yeast beta glucans with the β 1, 3-glucan backbone with β 1, 6-

glucan branches. Lentinan is purified from the mushroom *Lentinus edodes* (Shiitake) and is known to have antitumor properties and immunomodulatory activity on cell mediated and humoral immunity (Ina et al., 2013). Lentinan with > 80% purification was used in an *in vitro* study as a typical beta glucans source at 40, 80, and 160 µg/mL to examine its influence on broiler splenocyte proliferation, production of IL-2, and receptor signal transduction of splenocytes (Chen et al., 2003). The results of this study reported a significant increase in splenocyte proliferation and IL-2 liberation at any level other than the control. In addition, calcium ion and nitric oxide both had dose-dependent increased response. Only 80 and 160 µg/mL increased cAMP and cGMP after 20 and 60 min incubation. The authors indicated that the increase in IL-2 level, because of the Ca^{2+} concentrate elevation, could partially explain the increase in splenocyte proliferation. Yeast beta glucans supplement (Immustim, Immudyne, Houston, TX) was used in a broiler study either for 7 days or 25 days at 22 ppm of the diet, and both treatments were subdivided into 2 groups, one of them subjected to *E. coli* challenge via air-sac inoculation on day 7 (Huff et al., 2006). Both dietary supplementation programs affected body weight on day 25; however, in challenged groups, feeding the broilers glucans for 7 days prior to challenge improved body weight significantly. Although treatment effect was observed in feed-to-gain ratio in non-challenged groups, it was significantly improved when glucans were fed for 7 days and followed by pathogenic challenge than for the challenged control group. Intestinal integrity is very essential to obtain maximum feed utilization and is assessed based on villi height, the ratio of villi height to crypt depth, and goblet cell number. In addition, the colonization of pathogens in the

gastrointestinal tract impairs the histological structure and as a result impacts the digestibility and absorption of nutrients from the diet. In addition, pathogens increase the host's immune stress and thus influence the host's performance. Dietary β 1,3 / 1,6-glucan extracted from *Saccharomyces cerevisiae* and purified at 91.5% glucan was used at 100 ppm in a broiler diet to investigate its influence on intestinal integrity, *Salmonella* Typhimurium colonization in the liver and ceca, and sIgA in the jejunum (Shao et al., 2013). Challenging birds with *Salmonella* negatively impacted villi height, villus height to crypt depth ratio, and goblet cell count, and it increased colonization of this pathogen in the ceca. In addition, sIgA expression cells and sIgA secretion were significantly increased during pathogenic challenge while the tight junction proteins claudins and occludin, that regulate the function of the intestinal epithelial barrier, were significantly decreased. A diet that incorporates beta glucans significantly mitigated these adverse effects and diminished the damage of intestinal mucosal barrier by increasing claudins and occludin expression, number of goblet cells, and total sIgA production in the jejunum. In another study, yeast glucan (*Aureobasidium pullulan*) was injected into Peking ducks as a vaccine adjuvant with bovine serum albumin (BSA) to evaluate the immunological response as humoral or cell-mediated immunity (Tang et al., 2011). Although no influence of beta glucan was observed at the anti-BSA-IgG level, proliferation of blood mononuclear cells was significantly improved. These studies have demonstrated that beta glucans can have antitumor and immunomodulatory activities with bactericidal capability. Variation in these properties can be explained as the result of differences in source, molecular weight, polymer length, and degree of branching.

Mannoproteins

Another major component of YCW is mannoproteins which are O and N glycosylated polypeptides found on the outer surface of the YCW; they regulate permeability of the wall to solutes (Lipke and Ovalle, 1998). Mannoprotein complexes represent 40% of the yeast wall mass with mean molecular weight between 100-200 kDa, and have *N*-glycans consisting of α-linked mannose units with a long 1,6-α-linked backbone and short 1,2- and 1,3-α-linked side chains (Orlean, 1997). The mannoproteins complex can be obtained from YCW by hot citrate buffer extraction, sulfhydryl reagent liberation, and P-glucanases digestion (Orlean, 1997).

Mannose acts as a key in lock-and-key interactions with lectins, carbohydrate-binding proteins, which act as a lock, that are found on the surface of bacteria and thus inhibits enterobacteria that exhibit Type-1 fimbriae adhesiveness to the surface of host cells (Zopf and Roth, 1996). The anti-adhesive property of mannose reduces bacterial colonization and may increase microbial susceptibility to antibiotics (Zopf and Roth, 1996). The adhesion strength between mannose and binding proteins can be increased when the sugar molecules are linked to a polymer backbone to form polyvalent oligosaccharides, which are stronger antiadhesive macromolecules (Lees et al., 1994). However, this polyvalent complex is not recognized as a host molecule and could be toxic or immunogenic to the host (Zopf and Roth, 1996). Several strains of *E. coli*, *Salmonella typhimurium,* and *S. enteritidis* have been reported as able to agglutinate MOS *in vitro* (Spring et al., 2000).

The application of YCW extracted from *Saccharomyces cerevisiae* as a source of mannan-oligosaccharides in animal trials has been heavily investigated to assess their potential as alternatives to antibiotic growth promoters (Bozkurt et al., 2012; Che et al., 2011; Ghasemian and Jahanian, 2016; Jahanian and Ashnagar, 2015; Sohail et al., 2012; Spring et al., 2000; Tian et al., 2016; Xiao et al., 2012). However, YCW has other major components beside MOS such as glucans and protein that make it inaccurate to describe YCW products as MOS. Nevertheless, literature has linked several beneficial effects to the supplementation of MOS in the feed of different animal species in addition to the anti–adhesion characteristic. One of these benefits is the immunomodulatory effect of MOS that increases cecal IgA in rats, bile IgA and systemic IgG in turkeys, and neutrophils in dog and fish (Swanson et al., 2002). However, this study did not provide the purity of the MOS that was used and the same product was defined in another study as a product contains YCW fragments derived from *Saccharomyces cerevisiae* (Spring et al., 2000).

The availability of pure MOS at feasible cost to conduct *in vivo* studies has been a challenge. Therefore, studies have been using different sources of less purified MOS that may contain other components. In poultry, it was reported that supplementation of feed with YCW as a source of MOS improved animal performance and health. Spring et al. (2000) reported that adding YCW at 4000 ppm (YCW was added at this level to ensure sufficient concentration of MOS to control colonization of pathogens) as a source of MOS reduced *Salmonella* population in the ceca of 10-day-old broiler chicks.

Clostridium perfringens

Clostridium perfringens (Cp) is an anaerobic gram-positive spore forming rod-shaped bacteria ubiquitous in the environment and part of the normal gastrointestinal microbiota (Timbermont et al., 2009). The Cp pathogen produces about 17 toxins, and based on the subset of four important toxins (α, β, ε, and ι) that are produced by individual strains, Cp is classified into five toxinotyping classifications; A, B, C, D, and E (Freedman et al., 2015). Cp employees its exotoxins to obtain thirteen essential amino acids because it does not have genetic mechanism to produce them (Cooper and Songer, 2009). This bacterium is one of the most common foodborne illness causing pathogens in the United States and is estimated to cause approximately 1 million cases/year of foodborne illness (CDC, 2015). The estimated cost of foodborne illness due to Cp is about $342 million in the United States (ERS, 2013). In poultry, it is estimated that the global cost associated with necrotic enteritis (NE) due to Cp is approximately $2 billion (McReynolds et al., 2004).

Clostridium perfringens causes clinical and/or subclinical classes of NE in poultry. The clinical type is classified into acute and peracute forms that are both characterized by diarrhea, ruffled feathers, anorexia, depression, and occasional sudden death with no clinical signs; however, the peracute form mostly causes mortality of up to 50% of the flock within hours of the appearance of symptoms (Freedman et al., 2015). The subclinical form of necrotic enteritis is characterized by low mortality, declined production, little or no diarrhea, and decreased weight gain and feed efficiency due to the chronic damages to the gut mucosa that cause mal-digestion and mal-absorption

(Freedman et al., 2015; Van Immerseel et al., 2009). Type A of Cp is the most common cause of NE, and type C is less common (Cooper and Songer, 2009). There are several factors that can establish and intensify the Cp infection and exacerbate its impact on the birds, such as feed ingredients, feed form, protein and energy contents, animal protein level, secondary parasitic infection, and low intestinal pH (Cooper and Songer, 2009).

For decades, it was understood that α toxin produced by Cp type A was the main toxin that causes NE in chickens, due to the work of (Al-Sheikhly and Truscott, 1977). This study infused supernatant of crude alpha and theta toxins extracted from the broth cultures of Cp type A into the duodenum of 4-week-old male broilers. However, there was no clear explanation of the assumption that α toxin was the causative agent of NE, although it was part of crude supernatant toxins (Van Immerseel et al., 2009). Keyburn et al. (2006) reported that α toxin mutant is virulent just like the toxin itself, and thus the latter is an unnecessary factor in NE infection, especially as not all α toxin producing strains of Cp cause NE. Recent study has proposed a novel toxin called Necrotic Enteritis Toxin B-like (NetB), which is produced by Cp type A strains isolated from NE-positive chickens and is the most virulent toxin associated with NE in poultry (Keyburn et al., 2008). This toxin shares some similarities with amino acid sequences of β toxin from Cp at 38% identity and α toxin from *Staphylococcus aureus* at 31% identity. Another factor that was proposed to cause NE is the collagenolytic activity of matrix metalloproteinases (MMPs), proteolytic enzymes which have a destructive effect on the lamina propria, the extra-cellular matrix, and intercellular junctions which lead to enterocytes' necrotic death (Olkowski et al., 2008).

Chicken-specific peptides arrays

In eukaryotic cells, most of the signal transduction and other cellular processes such as transcription, cytoskeletal rearrangement, apoptosis, immune system functioning, and metabolism are controlled by protein kinases (Manning et al., 2002). Studying active kinase enzymes is crucial to understanding cellular function since they can demonstrate their influences on their corresponding substrates (Arsenault and Kogut, 2012). With more than 1000 predicted protein kinases in the vertebrate genome (Hunter, 1987), only 518 human protein kinases were identified, which constitute approximately 1.7% of human genes (Manning et al., 2002). Peptide arrays, antibody based array, and spectrophotometry techniques are among several other methodologies that are used for kinomics studies; however, peptide arrays have widely available reagents and exclusively deal with active kinase (Arsenault and Kogut, 2012). In kinomics, the principles of peptide arrays start with: 1) the employment of kinase target sites, usually 15 amino acids, peptides, made and printed onto array surface, 2) applying a sample of a mixture of purified kinase or cellular lysate as a source of active protein kinases on the array, 3) the active kinases identify their kinase target sites and phosphorylate the serine, threonine, or tyrosine residue on the target peptides sequence by using γ-phosphate group-added ATP, and 4) visualization the phosphorylation process (Arsenault and Kogut, 2012).

Peptide arrays have been employed to understand cellular signaling in different conditions such as describing reactions to specific singular ligands, describing changes in phosphorylation-mediated signal transduction in disease condition, or studying cancer

(Arsenault et al., 2011). Until a short time ago, the wealth of online public phosphorylation databases are particularly for humans and mice. Recently, species-specific peptide arrays have been developed to study the kinomics of other species such as bovine, ovine, and chicken (Arsenault and Kogut, 2012; Jalal et al., 2009). Chicken-specific peptide arrays have been used to study the skeletal muscle metabolic change over time after *Salmonella* Typhimurium challenge (Arsenault et al., 2013). This study reported significant changes in AMPK phosphorylation and the insulin/mammalian target of the rapamycin (mTOR) signaling pathway. Chicken-specific peptides have the advantages of eliminating the possibility of cross-reactivity between chicken kinases and human or mouse phosphorylation target sites, maximizing the interaction potential, and preventing failed interaction between peptide epitopes of other species and chicken proteins (Arsenault and Kogut, 2012).

CHAPTER II

INFLUENCE OF DIFFERENT YCW PREPARATIONS AND YCW COMPONENTS ON BROILER PERFORMANCE AND METABOLIC PATHWAYS

Introduction

Antimicrobials are employed at sub-therapeutic levels to suppress the growth of pathogenic bacteria and improve performance of farm food animals. However, the increased demand to limit or ban the use of antibiotics at sub-therapeutic levels in livestock feed accelerates the urge to explore versatile antibiotic alternatives. Those alternatives have demonstrated variabilities and inconstant results in their influences on animal health and performance. These fluctuated effects are being observed not only with different types but also within the same antibiotic alternative.

Yeast cell wall (YCW) of *Saccharomyces cerevisiae* is one of those alternatives that has been investigated *in vivo* and *in vitro* and used commercially in animal feed. Mannan oligosaccharide (MOS) and beta glucans are the main two components of YCW that have been thought to contribute to its beneficial effects on host health and performance (Shao et al., 2013; Spring et al., 2000). Studies have demonstrated a quadratic response to YCW in starter broiler (Fowler et al., 2015b) and laying hen performance (Hashim et al., 2013), and the 250 ppm level is proposed to be an optimum dose.

The structure of YCW consists of the polysaccharides glucan and mannan, which links to proteins to form mannoproteins, lipids and chitin. However, the percentage of each component varies due to differences in fermentation conditions and cultivation methods. In addition, more than 600 strains of *Saccharomyces cerevisiae* have been identified and categorized into three main groups according to their substrates as wine, beer, and bread (Legras et al., 2007). This variation could be one of the factors contributing to the different outcomes that have been reported in the literature.

Challenges of foodborne pathogens in food production animals have intensified with increased switching over to antibiotic-free products. *Clostridium perfringens* is one of those pathogens that causes about one million cases per year of foodborne illness in the United States as reported by the Centers for Disease Control and Prevention (2016). The Economic Research Unit of the USDA estimated the annual medical and premature death cost of foodborne disease due to *Clostridium perfringens* at more than $342 million dollars in 2013 (ERS, 2013).

In addition to human health hazard, this pathogen causes significant damages and cost to the food production animal industry. The economic cost associated with this pathogen in livestock animals is due to its negative impact on animal health and performance because of the secretion of its toxins that can cause necrotic enteritis at different clinical levels. Increased mortality, morbidity, and gut lesions are the main signs associated with clinical necrotic enteritis. The subclinical form of necrotic enteritis, on the other hand, is characterized by performance retardation due to male digestion and absorption (Yitbarek et al., 2012). Although these signs and characteristics of necrotic

enteritis are noticeable, there are other mechanisms in the background that can explain the long-term changes in the host.

Understanding these mechanisms can improve and accelerate strategies to develop or invent new antimicrobial alternatives to counter this pathogen. Kinomic studies have the potential to provide understanding of cellular biology and response to stimuli such as disease, condition, or treatment via consideration of phosphorylation-mediated signal transduction (Arsenault and Kogut, 2012). The peptide arrays technique examines the active kinases, which, in addition to the availability of the reagents, makes it better than other kinomics procedures such as mass spectrometry and antibody based arrays (Arsenault and Kogut, 2012). Cellular metabolic signaling using chicken species-specific peptide arrays was first introduced by Arsenault et al. (2013) to study the changes in skeletal muscle metabolism over time after *Salmonella* Typhimurium infection. Their study suggested that metabolism of skeletal muscle is influenced by systemic effects of this pathogen via AMPK phosphorylation, which can signal ATP production, and activity alterations as well as insulin/mTOR signaling pathway disruptions, which can alter glucose metabolism.

The purpose of our study was to evaluate the influence of different products of YCW on starter broiler performance. These products have different purification levels as well as different preparation techniques. The evaluation was conducted through monitoring applicable production performance attributes such body weight and feed conversion ratio. In addition, to help understand the mechanism of these changes in performance characteristics, a chicken species-specific peptide arrays technique was employed in this study.

Materials and Methods

Experimental design

This study was conducted to evaluate purified and semi-purified YCW preparations on starter broiler performance. A total of 240 Ross 308 (1-day-old) chicks were distributed between two battery brooder units (48 pens; 5 birds/pen). Six treatments were randomly assigned to pens and each treatment had eight replicates. A basal broiler starter diet (Table 2.1) was prepared and divided into five batches that included non-challenge (T1) and challenge (T2) control (NCh-Cont and Ch-Cont respectively), semi-purified YCW (250 ppm; SPYCW or T3), purified YCW (250 ppm; PYCW or T4), 50% purified beta-glucan YCW (130 ppm; BG or T5), and 99.9% purified MOS (53 ppm; MOS or T6). The YCW components were 23.5% MOS and 23% β-glucans. The SPYCW, PYCW, and BG preparations were provided by (Phileo-Lesaffre Animal Care, Milwaukee, WI) and MOS (≥ 99.7% purified from baker's *Saccharomyces cerevisiae*)

purchased from Sigma (Sigma-Aldrich, St. Louis, MO). Mortality within the first three days was replaced with new birds that had been fed on the control diet.

All birds of six treatments were challenged with infectious bursal disease vaccine (Schering Plough Animal Health, Millsboro, DE) on day 10. The vaccine was administered at 10x the manufacturer's recommended dose via the ocular route to immunocompromised the chicks (McReynolds et al., 2004). On day 16 and 17, all treatments were challenged with *Clostridium perfringens* (Cp) (10^7 cfu/mL, 3 mL oral gavage). Feed and water were provided ad libitum. Feed and body weight data were recorded on days 1, 10, 16 and 21. Performance index (PI) was calculated as: livability (%) x BW (kg)/Age (d)/FCRx100. The study was conducted at the Southern Plains Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture and was approved by the Texas A&M Institutional Animal Care and Use Committee and the Animal Care and Use Committee (ACUC) at the Southern Plains Agricultural Research Center.

Table 2.1 Composition and nutrient content of the basal starter diet for the yeast cell wall purification study.

Ingredient	Amount
Corn (%)	61.40
Dehulled soybean meal (%)	32.18
DL-Methionine 98% (%)	0.23
Lysine HCl (%)	0.18
Fat, blended animal/vegetable (%)	2.22
Limestone (%)	1.44
Mono-dicalcium phosphate (%)	1.55
Salt (%)	0.51
Trace minerals premix[1] (%)	0.05
Vitamin premix[2] (%)	0.25
Calculated nutrient content	
CP (%)	22.12
ME (Kcal/kg)	3050
Crude fat (%)	4.06
Crude fiber (%)	2.15
Calcium (%)	0.95
Available phosphate (%)	0.45
Sodium (%)	0.22
Methionine (%)	0.56
Lysine (%)	1.31

[1]Trace minerals premix added at this rate yields (mg/kg): zinc, 60.0; manganese, 60.0.

[2]Vitamin premix added at this rate yields (per kg): vitamin A, 11 kIU; vitamin D_3, 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; B_{12}, 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; B_6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

Isolation and administration of *Clostridium perfringens*

Clostridium perfringens (Cp) medium was provided by the ARS, Southern Plains Agricultural Research Center/USDA. The isolation and preparation of Cp were as described in McReynolds et al. (2004). The Cp pathogen was a combination of four field isolates type A from three different regions (Georgia, Texas, and Virginia). These isolates were cultured individually, combined, and administered to the birds. Then, about one gram of the GI contents of birds diagnosed with necrotic enteritis was taken in an anaerobic chamber and placed onto 10 mL of liquid thioglycollate medium (Becton Dickinson Co., Sparks, MD) and incubated for 24 h at 37ºC.

The bacteria were streaked on to *Brucella* blood agar plates (Anaerobic Systems, Morgan Hill, CA) using a 10-µL loop, and those plates were incubated for 24 h at 37ºC. To ensure purity, a single colony was removed from the plate and streaked onto *Brucella* blood agar. Bacteria were frozen with 20% glycerol at -80ºC, and when pathogenic challenge was needed, the bacteria were grown in a thioglycollate medium for 12 h prior to challenge. All treatments except non-challenged birds were challenged with 3 mL of Cp (10^7 cfu/mL) through oral gavage on days 16 and 17.

Intestinal tissue sampling

Tissue samples from the duodenum and jejunum of five birds per treatment were collected and sent to the Southern Plains Agriculture Research Center / USDA laboratory to conduct kinome analysis using the chicken-specific peptide arrays technique as described by Arsenault et al. (2013). Three birds of each treatment were euthanized with CO_2 and dissected to collect the samples. These samples were

immediately flash frozen with liquid nitrogen to maintain kinase enzymatic activity and shipped to the USDA lab.

Chicken-specific peptide arrays

This technique was conducted according to the methodology that was described by Arsenault et al. (2013). Briefly, 40 mg samples were obtained from the tissues collected above and homogenized by a hand-held Qiagen TissueRuptor (Valencia, CA, USA) in 100 uL of lysis buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl,1 mM EDTA, 1 mM Ethylene glycol tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Na3VO4, 1 mM NaF, 1 µg/mL leupeptin, 1 g/mL aprotinin and 1 mM phenylmethylsulphonyl fluoride (Sigma Aldrich, St. Louis, MO, USA). The following steps of the protocol were carried out as described by Arsenault et al. (2012): Cells were incubated on ice for 10 min and spun in a microcentrifuge for 10 min at 4°C. A 70-µL aliquot of this supernatant was mixed with 10 µL of activation mix (50% glycerol, 500 µM ATP [New England BioLabs, Pickering, ON, Canada], 60 mM $MgCl_2$, 0.05% [vol/vol] Brij 35, and 0.25 mg/mL bovine serum albumin [BSA]) and incubated on the array for 2 h at 37°C. Arrays were then washed with phosphate-buffered saline (PBS)–1% Triton.

Slides were submerged in phospho-specific fluorescent ProQ Diamond Phosphoprotein Stain (Invitrogen) with agitation for 1 h. Arrays were then washed three times in destaining solution containing 20% acetonitrile (EMD Biosciences, VWR Distributor, Mississauga, ON, Canada) and 50 mM sodium acetate (Sigma) at pH 4.0 for 10 min. A final wash was done with distilled deionized H_2O. Arrays were air dried for

20 min and then centrifuged at 300 × g for 2 min to ensure no moisture was remaining on the array.

Arrays were read using a GenePix Professional 4200A microarray scanner (MDS Analytical Technologies, Toronto, ON, Canada) at 532 to 560 nm with a 580-nm filter to detect dye fluorescence. Images were collected using GenePix version 6.0, software (MDS), and the spot intensity signal was collected as the mean of pixel intensity using local feature background intensity calculation with the default scanner saturation level.

Statistical analysis

Body weight, weight gain, and feed conversion ratio data were analyzed as a one-way ANOVA using the General Linear Model of SPSS software. Pen level was used as a block to control variation among groups of experimental units, and the design of the experiment was an incomplete block. Means deemed significant at $p \leq 0.05$ were separated using a protected Duncan's Multiple Range Test.

Peptide arrays data, images were gridded using GenePix pro software, and the spot intensity signal was collected as the mean of pixel intensity using local feature background intensity calculation with default scanner saturation level. The resultant data was then analyzed by the PIKKA2 peptide array analysis software. Briefly, the resulting data points were normalized to eliminate variance due to technical variation such as random variation in staining intensity between arrays or between array blocks within an array. Variance stabilization normalization was performed. (3 values/peptide). One-sided paired t-test between treatment and control values for a given peptide was conducted to calculate the p-value.

Results and Discussion

This experiment was conducted to assess the influence of different YCW preparations containing different concentrations of β-glucans or mannan oligosaccharides on starter broiler performance in the presence of microbial stress. Performance data were calculated and analyzed on days 10 (pre-vaccination), 16 (pre-challenge) and 21 (post-challenge). In addition, a chicken-specific peptide arrays technique was employed to study the mechanism of these YCW products in ameliorating the impact of Cp using samples collected on day.

Broiler performance

The 99% purified MOS treatment demonstrated a significant increase in body weight (BW) and weight gain (WG) on day 10 (Table 2.2), pre-vaccination, with an approximately 24-41 g difference from all other groups. No significant differences were observed between treatments in phase 1 feed-to-gain ratio (P-F:G), feed-to-body weight ratio (FCR), or phase mortality (P-Mort). There was no mortality observed in this phase in all treatments, except the MOS group had 5% mortality.

On day 16, pre-challenge and after birds been immunocompromised with a high dose of bursa vaccine, the 99% MOS treatment had significantly higher BW (559 g) than the semi-purified and 50% glucan treatments (490 and 513 g, respectively). The semi-purified YCW treatment exhibited significantly lower WG (246 g) than all other treatments except the 50% glucan treatment (263 g), and the latter was as good as the MOS group (274 g), but lower than the purified YCW, Ch-Cont, and NCh-Cont treatments (290, 291, and 301 respectively). The feed utilization was not efficient in

semi-purified treatment as well as 50% glucan and MOS treatments compared to other treatments. These three treatments had higher Phase 2 P-F:G (1.50, 1.50, and 1.54 respectively) and cumulative feed to gain ratio (C-F:G) (1.38, 1.39, and 1.38 consecutively) than purified YCW, Ch-Cont, and NCh-Cont treatments (1.31 for all last three treatments). The FCR scenario was not different from Phase 2 P-F:G or C-F:G except that semi-purified YCW treatment was not significantly different from Ch-Cont, NCh-Cont, or purified YCW treatments. The performance index (PI), which is based on BW, P-F:G, and mortality, was significantly lower in semi-purified YCW (222) and 50% glucan (232) groups than Ch-Cont (261), NCh-Cont (268) and purified YCW (261) treatments. The MOS treatment was not significantly different in PI (242) from Ch-Cont or Purified YCW, but was less than NCh-Cont. No mortality was recorded in phase 2 for all treatments (Table 2.3).

Table 2.2 Effect of yeast cell wall purification on broiler performance on day 10 (pre-immunocompromised).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	SPYCW	PYCW	BG	MOS
BW (g)	261 ± 19[b]	258 ± 34[b]	244 ± 14[b]	258 ± 27[b]	250 ± 19[b]	285 ± 20[a]
WG (g)	217 ± 17[b]	214 ± 34[b]	201 ± 14[b]	215 ± 27[b]	206 ± 20[b]	241 ± 19[a]
P-F:G	1.22 ± 0.04	1.21 ± 0.04	1.24 ± 0.05	1.19 ± 0.05	1.24 ± 0.06	1.20 ± 0.04
FCR	1.02 ± 0.03	1.00 ± 0.04	1.02 ± 0.05	0.99 ± 0.04	1.03 ± 0.05	1.01 ± 0.03
PI	214 ± 22	214 ± 22	197 ± 11	217 ± 24	201 ± 19	226 ± 29
P-Mort	0.0	0.0	0.0	0.0	0.00	5%

[a-b] Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; SPYCW = semi-purified yeast cell wall (YCW) at 250 pm; PYCW = purified YCW at 250 ppm; BG = 50% purified β-glucan at 130 ppm; MOS = 99.9% purified mannan-oligosaccharides at 53 ppm.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

BW = body weight; WG = weight gain; P-F:G = phase feed:gain; FCR = feed conversion ratio; PI = performance index; P-Mort = phase mortality.

Table 2.3 Effect of yeast cell wall purification on broiler performance on day 16 (pre-challenge).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	SPYCW	PYCW	BG	MOS
BW (g)	561 ± 29[a]	549 ± 52[ab]	490 ± 21[c]	549 ± 37[ab]	513 ± 32[bc]	559 ± 43[a]
WG (g)	301 ± 11[a]	291 ± 24[ab]	246 ± 18[d]	290 ± 16[ab]	263 ± 16[cd]	274 ± 29[cb]
P-F:G	1.38 ± 0.04[a]	1.40 ± 0.11[a]	1.50 ± 0.10[b]	1.41 ± 0.06[a]	1.50 ± 0.04[b]	1.54 ± 0.11[b]
C-F:G	1.31 ± 0.03[a]	1.31 ± 0.06[a]	1.38 ± 0.07[b]	1.31 ± 0.04[a]	1.39 ± 0.04[b]	1.38 ± 0.07[b]
FCR	1.21± 0.03[a]	1.21 ± 0.06[a]	1.26 ± 0.06[ab]	1.21 ± 0.04[a]	1.27 ± 0.04[b]	1.27 ± 0.06[b]
PI	268 ± 18[a]	261 ± 26[ab]	222 ± 16[c]	261 ± 19[ab]	232 ± 16[c]	242 ± 34[bc]
P-Mort	0.0	0.0	0.0	0.0	0.00	0.0
C-Mort	0.0	0.0	0.0	0.0	0.0	5%

[a-d] Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; SPYCW = semi-purified yeast cell wall (YCW) at 250 pm; PYCW = purified YCW at 250 ppm; BG = 50% purified β-glucan at 130 ppm; MOS = 99.9% purified mannan-oligosaccharides at 53 ppm.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

On day 21 (Table 2.4), post Cp challenge, birds in the NCh-Cont group demonstrated significantly higher BW (851 g), WG (289 g), and PI (274) than birds in the Ch-Cont group (that experienced 755 g, 206 g, and 199, respectively). The BW of the birds in the purified YCW (797 g) and MOS (805) groups was not significantly different from BW of NCh-Cont, but was significantly higher than semi-purified birds (712 g). The BW of 50% glucan birds (742 g) was significantly lower than NCh-Cont but was not significantly different from other treatments.

The phase 3 WG of the birds in both purified YCW (248 g) and MOS (247 g) treatments was not significantly different from NCh-Cont or all other treatments. However, the gain in body weight after the Cp challenge was significantly lower in semi-purified (222 g) or 50% glucan (232 g) treatment than the NCh-Cont group. Only the birds in purified YCW treatment demonstrated PI (238) not significantly different from NCh-Cont or other treatments. The PI of semi-purified YCW (203), 50% glucan (203), and MOS (202) treatments were significantly lower than NCh-Cont but were not different from Ch-Cont. No significant differences were observed between treatments in P-Mort and cumulative mortality (C-Mort).

Table 2.4 Effect of yeast cell wall purification on broiler performance on day 21 (post-challenge).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	SPYCW	PYCW	BG	MOS
BW (g)	851 ± 62[a]	755 ± 110[bc]	712 ± 38[c]	797 ± 62[ab]	742 ± 20[bc]	805 ± 72[ab]
WG (g)	289 ± 44[a]	206 ± 72[b]	222 ± 30[b]	248 ± 45[ab]	232 ± 35[b]	247 ± 41[ab]
P-F:G	1.75 ± 0.34	2.28 ± 0.50	1.92 ± 0.21	1.85 ± 0.13	2.00 ± 0.42	1.99 ± 0.55
C-F:G	1.45 ± 0.09	1.53 ± 0.07	1.55 ± 0.10	1.48 ± 0.04	1.55 ± 0.08	1.52 ± 0.12
FCR	1.38 ± 0.03[a]	1.44 ± 0.06	1.55 ± 0.10	1.39 ± 0.04	1.46 ± 0.08	1.43 ± 0.11
PI	274 ± 45[a]	199 ± 57[b]	203 ± 33[b]	238 ± 31[ab]	203 ± 32[b]	202 ± 77[b]
P-Mort	2.5%	15.0%	7.5%	7.5%	11.4%	18.1%
C-Mort	2.5%	15.0%	7.5%	7.5%	11.4%	22.5%

[a-c] Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; SPYCW = semi-purified yeast cell wall (YCW) at 250 pm; PYCW = purified YCW at 250 ppm; BG = 50% purified β-glucan at 130 ppm; MOS = 99.9% purified mannan-oligosaccharides at 53 ppm.

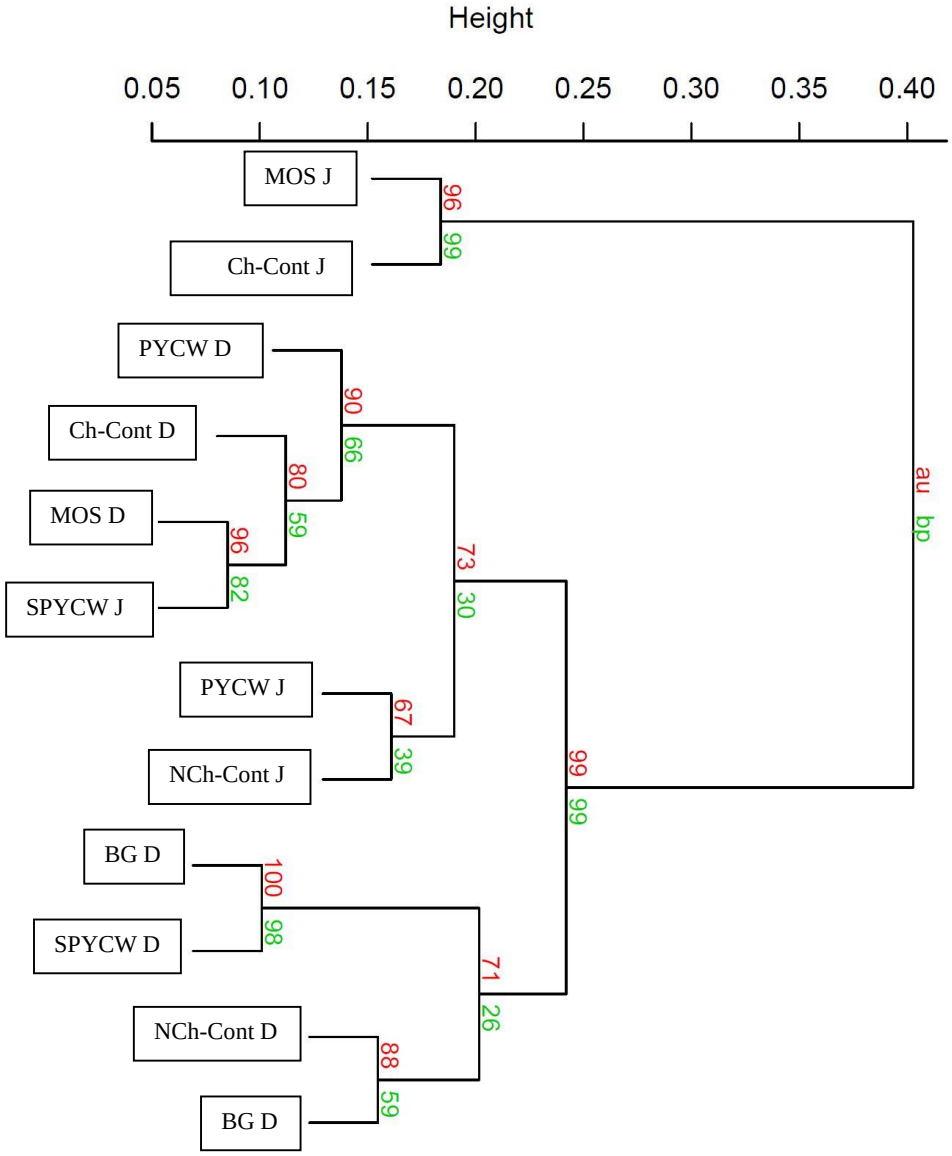
[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

Peptide arrays

On day 21, tissue samples were collected from three birds of each treatment from the duodenum and jejunum to study the chicken immune and metabolic signaling pathways using chicken-specific peptide arrays. The immune and metabolic pathways response in the duodenum and jejunum areas to the Cp challenge and YCW or its components was different (Figures 2.1 and 2.2). The results demonstrated that Cp challenge resulted in changes in the phosphorylation status of 367 peptides in the duodenum and 459 peptides in the jejunum of infected birds. The YCW wall or its components influenced changes in signaling pathways. In the duodenum, the percent of similarity coefficient (%CV) to NCh-Contr was 59.40% for glucan, 42.78% for semi-purified YCW, 33.97% for MOS, and 29.97% for purified YCW. In the jejunum, the purified YCW demonstrated higher similarity than other treatments. The CV% to NCh-Cont was 80.61% in purified YCW, 74.07% in semi-purified YCW, 69.06% in glucan, and 52.94% in MOS treatment. The signaling pathways of MOS and glucan are more like the purified YCW than like each other. In addition, the purified YCW was able to generate signaling that was not identical to either d by either of the YCW components alone.

Figure 2.1 Dendrogram of peptide arrays comparing similarity in metabolic pathways in the duodenum and jejunum of 21 days of age broilers.

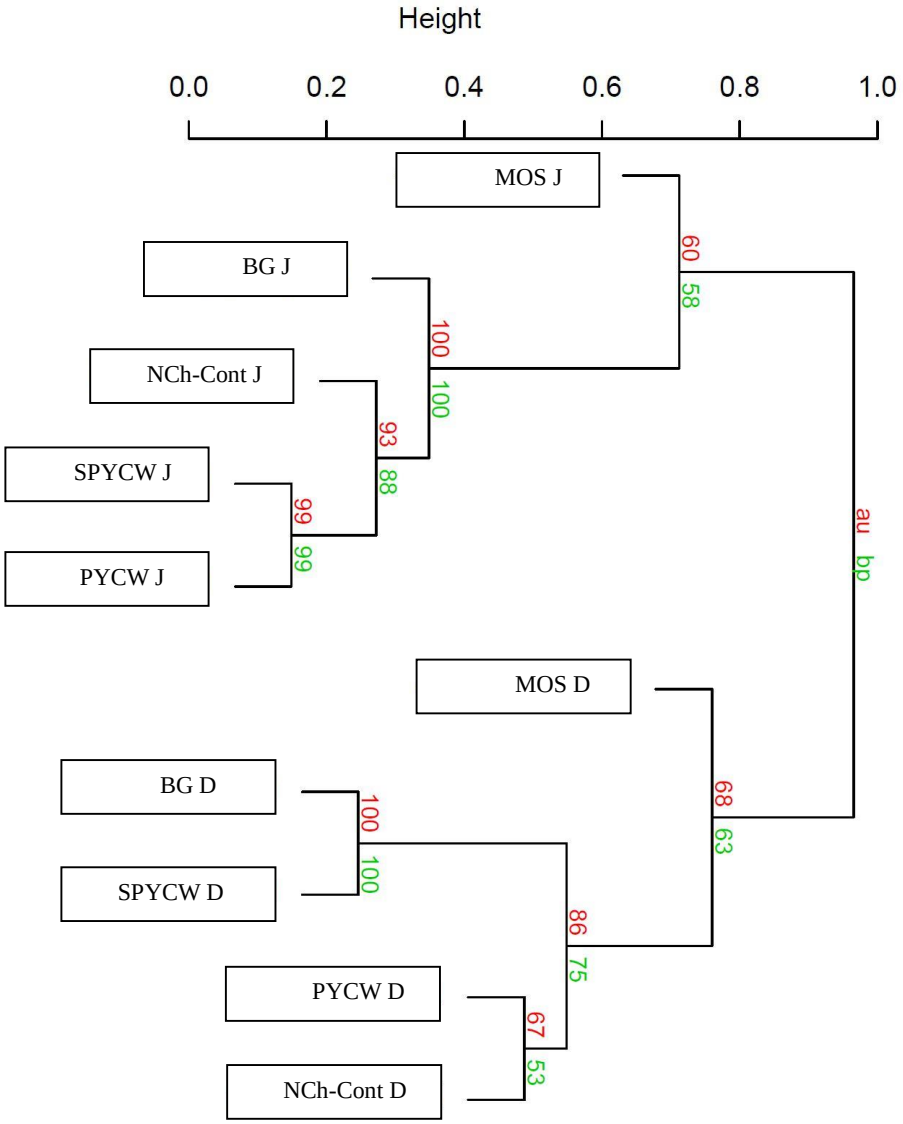


Relative similarity of band patterns is indicated by their grouping on the dendrogram and the percentage similarity coefficient (bar). NCh-Cont = non-challenged control; Ch-Cont = challenged control; SPYCW = semi-purified yeast cell wall (YCW) at 250 pm; PYCW = purified YCW at 250 ppm; BG = 50% purified β-glucan at 130 ppm; MOS = 99.9% purified mannan-oligosaccharides at 53 ppm.

NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

J = Jejunum, D = Duodenum.

Figure 2.2 Dendrogram of peptide arrays comparing similarity in metabolic pathways of mannan oligosaccharides vs challenged control in the duodenum and jejunum of 21 days of age broilers.



Relative similarity of band patterns is indicated by their grouping on the dendrogram and the percentage similarity coefficient (bar). NCh-Cont = non-challenged control; Ch-Cont = challenged control; SPYCW = semi-purified yeast cell wall (YCW) at 250 pm; PYCW = purified YCW at 250 ppm; BG = 50% purified β-glucan at 130 ppm; MOS = 99.9% purified mannan-oligosaccharides at 53 ppm.

NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens*

J = Jejunum, D = Duodenum.

The Cp challenge successfully impaired the starter broiler performance as demonstrated by BW, WG and PI reduction in control challenged birds on day 21 of age (after the Cp challenge). The application of YCW in broiler diets has been under heavy investigation with different nutritional, environmental, and pathological conditions or stresses. The goal of these type of studies was mainly to test the hypothesis whether YCW can be used as an alternative to the prophylactic use of antibiotics to promote growth in food production animals. The findings of this study reported that purified YCW, which is a typical commercial YCW product with typical MOS and glucan proportions, had no significant impact on broiler performance during the first 16 days of age under a controlled study environment. This is in agreement with the results of other researchers (Fowler et al., 2015b; M'Sadeq et al., 2015) who reported that the supplementation of YCW in broiler feed had no influence on performance in the early stage of age with no stressor. However, on day 21 of age and after Cp challenge, purified YCW was able alleviate the negative impact of Cp on broiler performance. Similar influence of YCW was reported in other studies when birds were subjected to Cp challenge (Fowler et al., 2015b; M'Sadeq et al., 2015). On the contrary, Yitbarek et al. (2012) reported that YCW could not improve BW in broiler starter birds challenged with Cp. However, their study supplemented the diet with 2000 ppm of YCW. Fowler et al. (2015b) stated that YCW has a quadratic effect on broiler performance and higher doses than 500 ppm could start to impair broiler performance.

Baker's YCW is a byproduct of *Saccharomyces cerevisiae* yeast extract production. Before YCW is obtained from yeast extract, the yeast itself undergoes

fermentation and maturation steps (ERUASYP, 2015). The fermentation occurs in large containers by adding sugar with sufficient oxygen supply at 30 °C. This is followed by concentration and washing steps in a centrifuge to remove the sugar residues and produce a creamy viscous yeast mass. The maturation of the later product is performed at 45-55 °C. When the temperature reaches 40 °C, yeast growth ceases, enzymes break down the yeast protein and part of the YCW disintegrates. To separate the remaining YCW from the yeast extract, a centrifugation step is performed. Therefore, the degree of purification of the final YCW product can vary. This study utilized purified and semi-purified YCW products. The latter included a byproduct of the yeast extract production process.

The performance of starter broiler birds significantly declined starting on day 16, after the immunocompromised challenge, and continued on day 21, post-pathogenic challenge. Other unpublished studies were conducted in our lab to evaluate the semi-purified YCW and the results were in agreement with the outcomes of the current study.

Glucan and MOS are the main polysaccharide components of YCW. Previous literature has used the terms MOS or glucan to describe YCW products and has referred to either one of them as the subject of their research. The availability of commercial pure MOS or glucan is cost prohibitive. Therefore, 99% purified MOS was obtained from Sigma to evaluate its influence on broiler performance and understand the mechanism of action. Glucan was provided as 50% purified from the YCW of *Saccharomyces cerevisiae*. Both of these components were included at levels equivalent to their proportion in YCW. The performance of the starter broiler birds was significantly

improved when the diet was supplemented with 99% MOS. This improvement was noticeable starting on day 10 in body weight. Moreover, the performance of the birds in the MOS treatment was not impacted by the immunocompromising high bursa vaccine dose. Addition of MOS to the diet increased the resistance of the birds to the impact of Cp on day 21, post-challenge, which was indicated by improved body weight and PI in MOS treatment birds to make them as good as non-challenged control birds. Addition of glucan purified at 50% of baker's YCW to broiler diet did not improve the performance of starter broiler birds. This may indicate that additional tests of this component at variable levels are needed.

In another study, glucan was extracted from *Saccharomyces cerevisiae* at 91% of the content of the extract and added to the diet of broilers infected with *Salmonella* (Shao et al., 2013). The study reported that 100 ppm of glucan improved intestinal integrity and immunity. The villus height, villus height:crypt depth ratio, goblet cell number, and sIgA expression cells and content in the jejunum were increased. In addition, *Salmonella* colonization in the ceca and invasion into the liver were inhibited. The study also reported that β-glucan improved the intestinal tight junction proteins in infected birds. Claudin-1 and occludin mRNA expression in the jejunum were improved in the *Salmonella* infected birds fed the diet supplemented with β-glucan. Tian et al. (2016) reported increased BW in broilers fed diet supplemented with 200 ppm of 91.5% pure yeast glucan between 21-42 days of age and on day 42 of the study. Improved phase FCR was also reported between 13-21 and 21-42 days of age, but not in the cumulative FCR. However, no significant improvement was observed in broiler

performance when those birds were challenged with Cp. Nevertheless, β-glucan improved villus height and crypt depth in challenged birds 7 days post challenge. The relative mRNA expression of endogenous antimicrobial peptides such as cathelicidin-1, cathelicidin-2, AvBD-1, AvBD-4, and AvBD-10 in the jejunum increased in glucan treatment birds post Cp challenge. Moreover, the Cp count in the cecum of infected glucan fed birds was decreased 14 and 21 days after challenge.

This is the first time that chicken-specific peptide arrays were employed to study the influence of YCW or its components, MOS and glucan, on performance of starter broilers subjected to Cp challenge. The peptide arrays results demonstrated that Cp challenge impacted the immune and metabolic pathways in the intestine. A total of 459 and 367 peptides in the jejunum and duodenum respectively that were significantly changed due to the Cp challenge. The YCW preparations or YCW components had different degree of influence on these peptides and this influence was also different in each part of the intestine. Whereas MOS and β-glucan treatments demonstrated only 52.94 and 69.06% similarity compared to NCh-Cont in the jejunum, PYCW and SPYCW treatments were 80.61 and 74.07% respectively. In the duodenum section, the similarity coefficient was less noticeable for all treatments and the β-glucan treatment still had higher similarity coefficient (59.40%) than MOS treatment (33.97%). This PYCW was able to generate signaling neither of component (β-glucan or MOS) was able to generate alone. This may indicate that YCW components have synergetic effect and can have better influence on broiler performance than each component alone.

CHAPTER III

ASSESSEMENT OF ALGAE BETA GLUCAN AND YEAST MANNAN ON BROILER PERFORMANCE UNDER CLOSTRIDIUM CHALLENGE

Introduction

Mannan and β-glucans are components of cell wall in many prokaryotic and eukaryotic organisms. β-glucans are polysaccharides consisting of glucose polymers and can be derived from different sources such as barley, fungi, yeast, bacteria, or algae. The structures of β-glucans are different between these sources (Jacob and Pescatore, 2014). While bacterial glucans consist of an unbranched 1,3-β-linked glycopyranosyl molecules backbone, yeast and fungal glucans have branched structures due to side chains joined to the backbone with 1,6-β-links. Glucans from algae (*Euglena gracilis*) are similar to bacterial glucan and consist of unbranched linear chains of glucose with 1,3-β-links. β-glucans have been attracting interest from researchers and industry for their beneficial influences on human and animal health. Examples of those benefits include immune-stimulatory, antimicrobial, anti-inflammatory, and antitumor properties (Zekovic et al., 2005).

Differences in the structure of β-glucans are linked to their different physiological functions (Jacob and Pescatore, 2014). Other factors also play significant roles in the biological activity of glucans such as degree of branching, solubility, molecular weight, polymer charge, and solution conformation (Zekovic et al., 2005). Most β-glucans that are used for research or commercial applications do not have high

purity (Novak and Vetvicka, 2009; Skov et al., 2012); they usually have 70% or less of purification, which might introduce other components to contribute to the immune-stimulatory effects. This purity issue contributes to contradictions in reported findings and makes it challenging to understand the mechanism of action of β-glucans (Novak and Vetvicka, 2009; Skov et al., 2012).

The Food and Drug Administration approved *Euglena gracilis* biomass containing 1,3-β-glucan as a generally recognized as safe (GRAS) product that can be used as a human food supplement (FDA, 2014). It was reported that 1,3-β-glucan derived from *Euglena gracilis* decreased mortality rate in mice infected with a lethal dose of *E. coli (Richard et al., 2013)* . In addition, specific (antibody titer) and non-specific (natural killer cell activity and phagocytosis activity) immune responses were improved. The researchers suggested that 1,3-β-glucan can serve as an adjuvant to vaccines due to its ability to enhance antibody titer. Another study reported significant improvement in FCR and reduction in *Eimeria* oocytes shedding in broilers fed diets supplemented with 50% purified algae glucan and challenged with *Eimeria* (Tian et al., 2016).

A mannan oligosaccharide is a complex of glucomannoproteins that can be derived from the inner cell wall of fungi (mushroom or yeast). The mannan in YCW is found as a complex with protein called mannoproteins complex which comprises 40% of the YCW mass (Lipke and Ovalle, 1998). The YCW typically contains 15-30% MOS (ERUASYP, 2015). YCW-derived mannan is a linear polymer of mannose sugar consisting of an α 1-6 linked backbone and α 1-2 and 1-3 linked branches. Brewer's

dried or baker's yeasts are the main source of MOS for commercial applications. Because of the high cost associated with pure MOS, gums or YCW products which contain mannose-based carbohydrates, have been used in research and commercial applications as a source of MOS (Spring et al., 2000; Xiao et al., 2012). This research with gums and YCW products has linked MOS as the sole dependent variable, although it is not the only component in those MOS source products.

One of the main effects that have been attributed to MOS is its ability to reduce the colonization of specific pathogenic microorganisms in the gut of the host. MOS inhibits colonization due to the attachment of bacteria exhibiting Type-1 fimbriae to the MOS which prevents bacterial attachment to the intestinal mucosa (Spring et al., 2000). In addition, supplementing broiler diets with YCW-derived MOS improves BW and FCR when the birds are subject to various environmental, nutritional, or pathological stressors (Benites et al., 2008; Hofacre et al., 2003; Sohail et al., 2012).

In this study, a linear unbranched 1,3-β-glucan from *Euglena gracilis* with ≥ 98% purity and MOS from *Saccharomyces cerevisiae* with ≥ 98% purity were evaluated in two separate studies at three levels. The dietary influence of these highly purified products on broiler performance was investigated under pathogenic challenge with *Clostridium perfringens* after the birds were immunocompromised with a high dose of bursal disease vaccine.

Materials and Methods

Two separate studies were conducted to evaluate different concentrations of a linear unbranched 1,3-β-glucan from *Euglena gracilis* (≥ 98% purity, Sigma-Aldrich, St. Louis, MO) or MOS from baker's yeast *Saccharomyces cerevisiae* (≥ 99% purity, Sigma-Aldrich, St. Louis, MO). In each study, these levels where compared with commercial baker's YCW (Safmannan, Phileo-Lesaffre Animal Care, Milwaukee, WI) product and two control groups either challenged with Cp or non-challenged (Ch-Cont and NCh-Cont). The Safmannan YCW is derived from baker's yeast of *Saccharomyces cerevisiae* and contains 23.5% mannoproteins and 23.8% β-glucans. The levels of high purity MOS and β-glucans were calculated based on their equivalent content in 250 ppm of YCW and two additional levels approximately 50% more or less than the equivalent level.

The studies were conducted at the Southern Plains Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture and were approved by the Texas A&M Institutional Animal Care and Use Committee and the Animal Care and Use Committee (ACUC) at the Southern Plains Agricultural Research Center.

Dietary treatments

In both studies, a basal corn/soy based broiler starter diet (Table 3.1) was formulated to meet the nutrient requirements suggested by the Ross-308 management handbook. The diet was then divided into five batches that included one batch for non-challenge (T1) and challenge (T2) control, one for YCW (250 ppm; T3), and three other

treatments that are supplemented with different levels of either high purity glucan or MOS.

The other three treatments in the β-glucan study included β-glucan at 30 ppm (T4), 60 ppm (T5), and 90 ppm (T6). For the MOS study, the other three treatments included MOS at 25 ppm (T4), 50 ppm (T5), and 100 ppm (T6).

Animal husbandry

In each study, a total of 240 Ross 308 1-day-old chicks were distributed between two battery brooder units (48 pens; 5 birds/pen). Six treatments were randomly assigned to pens and each treatment had 8 replicates. All birds of 6 treatments were vaccinated with infectious bursal disease vaccine (Schering Plough Animal Health, Millsboro, DE) on day 10. The vaccine was administered at 10x the manufacturer's recommended dose via the ocular route in order to immunocompromised the chicks (McReynolds et al., 2004). On day 16 and 17, all treatments were challenged with Cp (10^7 cfu/mL, 3 ml oral gavage). Mortality within the first three days was replaced with new birds that had been fed the control diet. Feed and water were provided ad libitum. Feed and body weight data were recorded on days 1, 10, 16 and 21. The isolation and administration of Cp was performed as described previously in chapter II.

Table 3.1 Composition and nutrient content of the basal starter diet for algae beta glucan and yeast mannan oligosaccharides.

Ingredient	**Amount**
Corn (%)	61.40
Dehulled soybean meal (%)	32.18
DL-Methionine 98% (%)	0.23
Lysine HCl (%)	0.18
Fat, blended animal/vegetable (%)	2.22
Limestone (%)	1.44
Mono-dicalcium phosphate (%)	1.55
Salt (%)	0.51
Trace minerals premix[1] (%)	0.05
Vitamin premix[2] (%)	0.25
Calculated nutrient content	
CP (%)	22.12
ME (Kcal/kg)	3050
Crude fat (%)	4.06
Crude fiber (%)	2.15
Calcium (%)	0.95
Available phosphate (%)	0.45
Sodium (%)	0.22
Methionine (%)	0.56
Lysine (%)	1.31

[1]Trace minerals premix added at this rate yields (mg/kg): zinc, 60.0; manganese, 60.0.

[2]Vitamin premix added at this rate yields (per kg): vitamin A, 11 kIU; vitamin D_3, 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; B_{12}, 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; B_6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

Statistical analysis

For each study, body weight, weight gain, and feed conversion ratio data were analyzed as a one-way ANOVA using the General Linear Model of SPSS software. Pen level was used as a block to control variation among groups of experimental units and the design of the experiment was an incomplete block. Means deemed significant at $p \leq 0.05$ were separated using a protected Duncan's Multiple Range Test.

Results and Discussion

Two separate studies were conducted to evaluate the influence of different levels of highly purified unbranched 1,3-β-glucan from the algae *Euglena gracilis* (≥ 98.5 purity) and MOS from baker's yeast *Saccharomyces cerevisiae* (≥ 99.9% purity) on immunocompromised broiler performance under pathogenic challenge stress. The levels of both polysaccharides were compared with challenged and non-challenged control groups as well as challenged YCW treatment. In both studies, birds were immunocompromised on day 10 by administering the birds via ocular route with 10x the recommended level of commercial bursal disease vaccine (Fowler et al., 2015b; McReynolds et al., 2004). On day 16 and 17, birds in all treatments, except the non-challenged control group, were subjected to Cp challenge by orally gavage the birds with 3 ml of 10^7 cfu of Cp/mL.

For the glucan study, there were no significant differences between treatments in broiler performance on day 10 (Table 3.2), pre-vaccination (phase 1) . On day 16 (phase 2), post-immunocompromising with bursal disease vaccine, all glucan treatments demonstrated the trend of improved performance. Specifically, birds fed a diet supplemented with 60 ppm of 1,3-β-glucan demonstrated significantly higher BW (542 g) and WG (291 g) than birds in NCh-Cont (506 and 253 g, respectively) and Ch-Cont (497 and 249 g, respectively) treatments. No significant differences were observed in BW and WG between all glucan and YCW treatments. The birds in the 60 ppm glucan treatment had the lowest P-F:G (1.30) and C-F:G (1.26) and were significantly lower than BG-30 (1.38 and 1.32) and NCh-Cont (1.40 and 1.31). The P-F:G and C-F:G in BG-90 (1.35 and 1.29) were not significantly different from any of the other glucan treatments, either control groups, or YCW treatment (P-F:G = 1.33 and C-F:G = 1.29 for all treatments). No significant differences were observed in FCR or PI between treatments (Table 3.3).

Table 3.2 Effect of algae beta glucan levels on broiler performance on day 10 (pre-immunocompromised).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	BG-30	BG-60	BG-90
BW (g)	253 ± 12	248 ± 9	242 ± 18	256 ± 20	251 ± 17	252 ± 10
WG (g)	208 ± 12	202 ± 9	197 ± 18	210 ± 20	207 ± 17	207 ± 10
P-F:G	1.21 ± 0.03	1.20 ± 0.04	1.23 ± 0.04	1.25 ± 0.05	1.20 ± 0.03	1.21 ± 0.03
FCR	0.99 ± 0.03	0.98 ± 0.04	1.00 ± 0.03	1.02 ± 0.04	1.00 ± 0.02	1.00 ± 0.03
PI	204 ± 25	201 ± 17	192 ± 25	180 ± 31	204 ± 24	203 ± 21
P-Mort	2.5%	2.5%	2.5%	12.5%	2.5%	2.5%

Means ± SD

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; BG-30, BG-60, and BG-90 = 98.3% purified Algae β-glucan at 30, 60, and 90 ppm respectively.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; PI = performance index; P-Mort = phase mortality.

Table 3.3 Effect of algae beta glucan levels on broiler performance on day 16 (pre-challenge).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	BG-30	BG-60	BG-90
BW (g)	506 ± 32^{bc}	497 ± 21^{c}	510 ± 36^{bc}	532 ± 35^{ab}	542 ± 37^{a}	519 ± 16^{abc}
WG (g)	253 ± 24^{bc}	249 ± 20^{c}	267 ± 19^{abc}	276 ± 28^{ab}	291 ± 26^{a}	267 ± 16^{abc}
P-F:G	1.40 ± 0.04^{c}	1.35 ± 0.04^{abc}	1.33 ± 0.03^{ab}	1.38 ± 0.08^{bc}	1.30 ± 0.05^{a}	1.35 ± 0.04^{abc}
C-F:G	1.31 ± 0.03^{b}	1.28 ± 0.03^{ab}	1.29 ± 0.06^{ab}	1.32 ± 0.05^{b}	1.26 ± 0.03^{a}	1.29 ± 0.02^{ab}
FCR	1.19 ± 0.04	1.16 ± 0.03	1.17 ± 0.05	1.20 ± 0.04	1.16 ± 0.03	1.17 ± 0.03
PI	236 ± 30	237 ± 21	243 ± 34	215 ± 40	255 ± 22	245 ± 22
P-Mort	0.0	0.0	0.0	3.1%	2.5%	0.0
C-Mort	2.5%	2.5%	2.5%	15.0%	5.0	2.5%

[a-c] Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; BG-30, BG-60, and BG-90 = 98.3% purified Algae β-glucan at 30, 60, and 90 ppm respectively.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

On day 21 (phase 3, Table 3.4), post-challenge, the pathogenic challenge with Cp significantly impacted the Ch-Cont, as observed through lower BW (706 g) and WG (206 g) compared with NCh-Cont (783 and 277 g, respectively). Supplementing broiler starter diet with 1,3-β-glucan improved broiler resistance to the negative impact of Cp on BW and WG. The BW of the birds in BG-30 (784 g), BG-60 (777 g), and BG-90 (770 g) treatments was significantly higher than BW of Ch-Cont birds. However, no significant difference was observed in BW between these glucan treatments and NCh-Cont. The BW of YCW birds (752 g) was not significantly different from any of the glucan treatments or any of the control groups. A similar scenario was observed in WG except that birds in the BG-60 (235 g) treatment were not significantly different from Ch-Cont. However, no significant difference in WG was observed between BG-60 and both BG-30 (253 g) and BG-90 (251 g), and the latter treatment had significantly higher WG than the Ch-Cont group. The WG of birds in the YCW treatment (243 g) was not significantly different from any of the glucan treatments or the control groups. No significant differences were noted in any of the other performance variables such P-F:G, C-F:G, FCR, PI, or mortality. The dose effect of BG on BW is presented in Figure 3.1 (BW was fit to the BG dose in a broken-line quadratic model; $R2 = 0.288$). The maximum dose of BG required to have maximum BW was calculated to be no more than 27.98 ppm.

Table 3.4 Effect of algae beta glucan levels on broiler performance on day 21 (post-challenge).

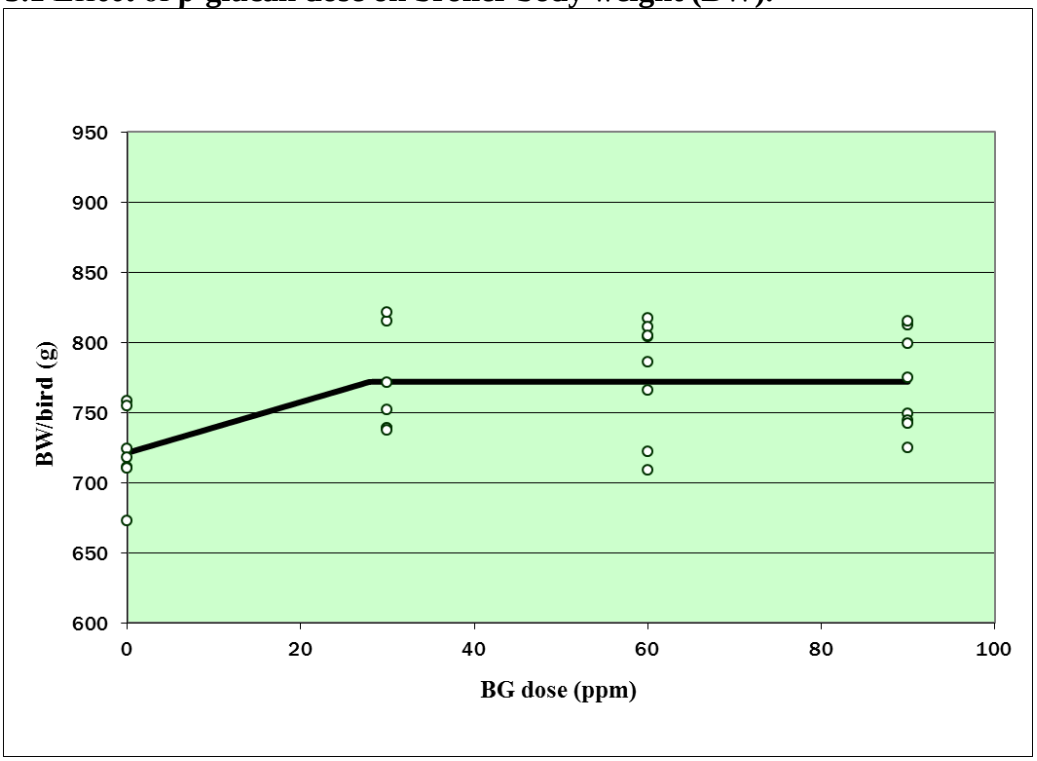
	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	BG-30	BG-60	BG-90
BW (g)	783 ± 61[a]	706 ± 51[b]	752 ± 70[ab]	784 ± 57[a]	777 ± 42[a]	770 ± 16[a]
WG (g)	277 ± 34[a]	209 ± 38[c]	243 ± 41[abc]	252 ± 34[ab]	235 ± 20[bc]	251 ± 32[ab]
P-F:G	1.54 ± 0.07	1.76 ± 0.19	1.67 ± 0.16	1.73 ± 0.12	1.80 ± 0.23	1.69 ± 0.15
C-F:G	1.40 ± 0.02	1.42 ± 0.04	1.41 ± 0.06	1.45 ± 0.04	1.41 ± 0.05	1.42 ± 0.04
FCR	1.31 ± 0.03	1.33 ± 0.04	1.32 ± 0.06	1.35 ± 0.03	1.33 ± 0.05	1.33 ± 0.04
PI	256 ± 51	225 ± 31	248 ± 36	213 ± 45	235 ± 38	245 ± 23
P-Mort	3.1%	2.5%	0.0	3.1%	5.5%	2.5%
C-Mort	5.0%	5.0%	2.5%	17.5%	10.0%	5.0%

[a-c] Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; BG-30, BG-60, and BG-90 = 98.3% purified Algae β-glucan at 30, 60, and 90 ppm respectively.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

Figure 3.1 Effect of β-glucan dose on broiler body weight (BW).



The β-glucan (BG) dose was calculated to be no more than 27.98 ppm to have maximum body weight (BW) on day 21.

The results of highly purified MOS (≥ 99.9% purity) extracted from the baker's yeast *Saccharomyces cerevisiae* study demonstrated no significant differences in starter broiler performance between treatments on day 10, pre-vaccination, or day 16, pre-challenge (Tables 3.5 and 3.6).

Table 3.5 Effect of yeast mannan oligosaccharides levels on broiler performance on day 10 (pre-immunocompromised).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	MOS-25	MOS-50	MOS-100
BW (g)	273 ± 9	264 ± 10	264 ± 7	259 ± 14	265 ± 6	270 ± 15
WG (g)	230 ± 8	221 ± 11	221 ± 7	216 ± 14	222 ± 6	227 ± 14
P-F:G	1.14 ± 0.02	1.13 ± 0.01	1.15 ± 0.03	1.17 ± 0.02	1.16 ± 0.03	1.16 ± 0.03
FCR	0.95 ± 0.02	0.94 ± 0.01	0.96 ± 0.02	0.97 ± 0.03	0.97 ± 0.02	0.97 ± 0.02
PI	241 ± 10	234 ± 11	225 ± 18	216 ± 21	229 ± 9	235 ± 19
P-Mort	0.0	0.0	2.5%	2.5%	0.0	0.0

Means ± SD.

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; MOS-25, 50, and 100 = 99.8% purified baker's yeast MOS at 25, 50 and 100 ppm respectively.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; PI = performance index; P-Mort = phase mortality.

Table 3.6 Effect of yeast mannan oligosaccharides levels on broiler performance on day 16 (pre-challenge).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	MOS-25	MOS-50	MOS-100
BW (g)	544 ± 42	544 ± 35	534 ± 49	529 ± 35	521 ± 60	552 ± 36
WG (g)	271 ± 44	280 ± 28	269 ± 47	270 ± 24	256 ± 59	282 ± 23
P-F:G	1.46 ± 0.32	1.34 ± 0.04	1.45 ± 0.21	1.41 ± 0.06	1.49 ± 0.34	1.38 ± 0.06
C-F:G	1.30 ± 0.12	1.25 ± 0.02	1.30 ± 0.08	1.30 ± 0.03	1.31 ± 0.10	1.28 ± 0.04
FCR	1.19 ± 0.10	1.15 ± 0.02	1.20 ± 0.06	1.19 ± 0.02	1.20 ± 0.08	1.18 ± 0.03
PI	266 ± 38	266 ± 33	244 ± 37	237 ± 43	245 ± 43	264 ± 35
P-Mort	0.0	2.5%	2.5%	5.0%	2.5%	2.5%
C-Mort	0.0	2.5%	5.0%	7.5%	2.5%	2.5%

Means ± SD.

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; MOS-25, 50, and 100 = 99.8% purified baker's yeast MOS at 25, 50 and 100 ppm respectively.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

However, on day 21, post-challenge, significant differences were noticed in BW and WG of Ch-Cont and NCh-Cont birds (Table 3.7). The BW of Ch-Cont birds (784 g) was significantly lower than BW of NCh-Contr birds (844 g). Nevertheless, none of the treatments improved body weight significantly. The WG was significantly lower in Ch-Cont (240 g) than NCh-Cont (300 g) treatment. Only the YCW treatment had WG (271 g) that was not significantly different from the NCh-Cont or Ch-Cont group. No significant difference was reported in WG between MOS-25 (251 g), MOS-50 (254 g), or MOS-100 (230 g) treatments. In fact, only the MOS-100 treatment had significantly lower WG compared to NCh-Cont or YCW treatments.

Although no significant difference was observed in P-F:G between treatments, MOS-100 birds had higher P-F:G (1.96) compared with NCh-Cont birds (1.51) at a p-value of 0.055 (Table 3.7). This MOS treatment also had significantly higher C-F:G and FCR (1.45 and 1.37 respectively) than NCh-Cont (1.36 and 1.29) and Ch-Cont birds (1.38 and 1.30). However, there were no significant differences in C-F:G and FCR between MOS-25 (1.43 and 1.34), MOS-50 (1.42 and 1.33), YCW (1.44 and 1.35) and MOS-100 treatments. The birds in the MOS-50 treatment were as good as NCh-Cont with respect to FCR. There was no significant difference in PI between treatments for any of the MOS or YCW birds, but the NCh-Cont had significantly higher PI than all of these treatments.

Table 3.7 Effect of yeast mannan oligosaccharides levels on broiler performance on day 21 (post-challenge).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	MOS-25	MOS-50	MOS-100
BW (g)	844 ± 29[a]	784 ± 29[b]	798 ± 47[b]	780 ± 36[b]	775 ± 40[b]	782 ± 59[b]
WG (g)	300 48[a]	240 ± 38[bc]	271 ± 29[ab]	251 ± 27[bc]	254 ± 44[bc]	230 ± 32[c]
P-F:G[3]	1.51 ± 0.17	1.71 ± 0.22	1.78 ± 0.29	1.72 ± 0.18	1.74 ± 0.35	1.96 ± 0.34
C-F:G	1.36 ± 0.03[a]	1.38 ± 0.05[ab]	1.44 ± 0.05[bc]	1.43 ± 0.03[bc]	1.42 ± 0.06[bc]	1.45 ± 0.07[c]
FCR	1.29 ± 0.03[a]	1.30 ± 0.05[ab]	1.35 ± 0.05[bc]	1.34 ± 0.03[bc]	1.33 ± 0.06[abc]	1.37 ± 0.06[c]
PI	281 ± 33[a]	251 ± 34[ab]	212 ± 56[b]	229 ± 44[b]	223 ± 45[b]	214 ± 54[b]
P-Mort	5.0%	5.6%	15.7%	5.6%	12.5%	15.0%
C-Mort	5.0%	7.5%	20.0%	12.5%	15.0%	17.5%

[a-c]Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; MOS-25, 50, and 100 = 99.8% purified baker's yeast MOS at 25, 50 and 100 ppm respectively.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

[3]p-value = 0.055.

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

In the glucan study, all levels of beta glucan demonstrated significantly improved broiler performance after immunocompromising on day 16 and after the Cp challenge on day 21. To the best of our knowledge, no published study has been conducted to assess the influence of the unbranched 1,3-β-glucan derived from *Euglena gracilis* (≥98.5 purity) or MOS derived from *Saccharomyces cerevisiae* (≥ 99.9% purity) on broiler starter under Cp challenge. However, Levine et al. (2015) evaluated the anticoccidial influence of two commercial feed additives (Algamune™AM or Algamune™ ZPC) that contain 50% wt 1,3-β-glucan derived from *Euglena gracilis* fed to broilers challenged with three *Eimeria spp.*. The authors reported significant improvement in FCR in birds that received 50 or 100 g/MT (25 or 50 ppm) of Algamune AM or 200 g/MT (100 ppm) of Algamune ZPC, which contains 2% zinc polysaccharide complex. In addition, 200 g/MT of Algamune AM or 100 g/MT of Algamune ZPC significantly reduced oocytes per gram of fecal material. Several studies have been conducted to investigate the effects of β-glucan derived from *Saccharomyces cerevisiae* on broiler performance, gut histology, and the immune system (Cox et al., 2010a; Cox et al., 2010b; Tian et al., 2016).

Nevertheless, the structures of yeast glucan and algae glucan are not very similar since the former is composed of 1,3-β-links with 1,6-β-links branches. No significant influence was reported in broiler performance when yeast β-glucan was added to the diets at 200 or 1000 ppm with or without pathogenic stress (Cox et al., 2010a; Cox et al., 2010b). However, Cox et al. (2010b) reported reduction in lesion scores due to *Eimeria* infection and enhancement in the immune response and improved T helper cells. Tian et

al. (2016) reported improved broiler performance, intestinal morphology, and immune response in broilers subjected to *Eimeria* challenge followed by Cp challenge.

In MOS study, the results demonstrated no significant improvement in broiler performance when fed diet supplemented with three levels of high purity baker's yeast MOS. However, a previous study reported improved performance in broilers fed 50 ppm MOS and subjected to Cp challenge (Chapter II). Other studies have reported improved broiler performance when fed diets supplemented with YCW derivatives as a source of MOS (Fowler et al., 2015a; Fowler et al., 2015b; M'Sadeq et al., 2015). However, MOS is not the only component of YCW, and results cannot be credited solely to MOS in these studies.

In conclusion, the supplementation of broiler diets with high purity β-glucan extracted from *Euglena gracilis* may have beneficial effect on reducing the impact of Cp challenge. This ameliorative influence can be noticed at a level not higher than 25 ppm. Addition of high purity MOS extracted from cell wall of baker's *Saccharomyces cerevisiae* to broiler feed did not demonstrate significant effect in elevating the impact of Cp challenge.

CHAPTER IV

INFLUENCES OF *SACCHAROMYCES CEREVISIAE*-DERIVED B-GLUCAN AND MANNAN ON BROILER PERFORMANCE

Introduction

β-glucan and mannan oligosaccharides (MOS) have been derived from different organisms such as yeast and algae. These two polysaccharides exist in different structures, depending on the links between the sugar polymers. Thus, their physiological functions can differ. This variation in their biological influence can cause different outcomes and interpretation in the literature. Understanding the effect of these two sugar polymers can be improved by using a single source to derive these polysaccharides.

Yeast cell wall (YCW) from *Saccharomyces cerevisiae* is one of the main sources to extract MOS and β-glucan for laboratory and industry applications. The structure of YCW is composed mainly of branched 1,3-β-glucan with 1,6-β-links, mannoproteins complex, and a negligible component of chitin (Lipke and Ovalle, 1998). Commercial YCW contains 30-60% polysaccharides including mannan and β-glucan polymers, 15-30% proteins, 5-20% lipids, and a small portion of chitin (ERUASYP, 2015). The MOS and most of the proteins are linked to form mannoprotein complexes.

The use of YCW in food production animal feed and in poultry specifically as an alternative to antibiotic growth promotors has been under investigation for decades (Fowler et al., 2015a; Fowler et al., 2015b; Ghosh et al., 2007; Hashim et al., 2013; M'Sadeq et al., 2015; Sohail et al., 2012; Spring et al., 2000). Several benefits from

supplementing poultry diets with YCW have been reported such as improvements in performance, digestive tract integrity, health status, and resistance to specific pathogens.

Since YCW products contain blends of polysaccharides (glucan and MOS) with significant amounts of proteins and lipids, it is not justified to describe YCW products as only MOS or only glucan. Therefore, this study aimed to study the influence of each polysaccharide component of YCW individually and in combination. To minimize the influence of different sources of MOS or glucan, these two components were derived from the same YCW product of *Saccharomyces cerevisiae*. The final purification of each component was obtained at a level that can practically be commercially available.

Materials and Methods

Dietary treatments

The YCW preparation was derived from baker's *Saccharomyces cerevisiae* (Phileo-Lesaffre Animal Care, Milwaukee, WI). This YCW consist of 23.8% glucans, 23.5% mannoproteins. The β-glucan was extracted from the same YCW and purified to be 50-55% β-glucan. The MOS was also derived from the same YCW to contain 50% mannoproteins.

A basal corn/soy based broiler starter diet (Table 4.1) was formulated to meet the nutrient requirements suggested by the Ross-308 management handbook. The diet was then divided into five batches that included one batch for non-challenge (T1) and challenge (T2) control, one for YCW (250 ppm; T3), MOS (117.3 ppm, T4), glucan (108.4 ppm, T5), and MOS+Glucan (117.3 + 108.4 ppm, respectively). The

concentrations of MOS and glucan were calculated to be equal to the amount of each one in the YCW treatment.

Animal husbandry

A total of 240 Ross 308 (1-day-old) chicks were distributed between two battery brooder units (48 pens; 5 birds/pen). Six treatments were randomly assigned to pens, and each treatment had eight replicates. All birds of the six treatments were challenged with infectious bursal disease vaccine (Schering Plough Animal Health, Millsboro, DE) on day 10. The vaccine was administered at 10x the manufacturer's recommended dose via the ocular route in order to immunocompromised the chicks (McReynolds et al., 2004). On day 16 and 17, all treatments were challenged with *Clostridium perfringens* (Cp) (10^7 cfu/mL, 3 mL oral gavage). Mortality within the first three days was replaced with new birds that had been fed the control diet. Feed and water were provided ad libitum. Feed and body weight data were recorded on days 1, 10, 16 and 21. The isolation and administration of Cp was performed as described previously in Chapter II.

Table 4.1 Composition and nutrient content of the basal starter diet for the yeast derived mannoproteins and β-glucan study.

Ingredient	**Amount**
Corn (%)	61.40
Dehulled soybean meal (%)	32.18
DL-Methionine 98% (%)	0.23
Lysine HCl (%)	0.18
Fat, blended animal/vegetable (%)	2.22
Limestone (%)	1.44
Mono-dicalcium phosphate (%)	1.55
Salt (%)	0.51
Trace minerals premix[1] (%)	0.05
Vitamin premix[2] (%)	0.25
Calculated nutrient content	
CP (%)	22.12
ME (Kcal/kg)	3050
Crude fat (%)	4.06
Crude fiber (%)	2.15
Calcium (%)	0.95
Available phosphate (%)	0.45
Sodium (%)	0.22
Methionine (%)	0.56
Lysine (%)	1.31

[1]Trace minerals premix added at this rate yields (mg/kg): zinc, 60.0; manganese, 60.0.

[2]Vitamin premix added at this rate yields (per kg): vitamin A, 11 kIU; vitamin D_3, 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; B_{12}, 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; B_6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

The study was conducted at the Southern Plains Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture and were approved by the Texas A&M Institutional Animal Care and Use Committee and the Animal Care and Use Committee (ACUC) at the Southern Plains Agricultural Research Center.

Statistical analysis

Body weight (BW), weight gain (WG), phase and cumulative feed to gain ratios (P-F:G and C-F:G), feed conversion ratio (FCR), and performance index (PI) data were analyzed as a one-way ANOVA using the General Linear Model of SPSS software. Outlier values were excluded from treatments T2 and T3. Because of unequal numbers of replicates per treatment, type I error was not guaranteed, and thus Duncan's and Tukey HSD tests were used to separate means.

Results and Discussion

The results at day 10 are presented in table 4.2. On day 10, pre-vaccination stress, there was no statistically significant difference between treatments for any of the measured variables. On day 16, pre-challenge with Cp, there was no significant difference in BW, WG or PI. However, diets supplemented with YCW or any of its components individually or combined improved P-F:G significantly.

Table 4.2 Effect of yeast cell wall components on broiler performance on day 10 (pre-immunocompromised).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	BG	MOS	BG+MOS
BW (g)	265 ± 15	255 ± 18	260 ± 9	260 ± 14	249 ± 20	257 ± 10
WG (g)	220 ± 15	210 ± 18	215 ± 9	214 ± 14	203 ± 20	212 ± 9
P-F:G	1.19 ± 0.04	1.19 ± 0.02	1.19 ± 0.02	1.19 ± 0.03	1.19 ± 0.04	1.19 ± 0.03
FCR	0.98 ± 0.02	0.98 ± 0.03	0.98 ± 0.02	0.98 ± 0.02	0.98 ± 0.03	0.98 ± 0.02
PI	218 ± 22	215 ± 13	219 ± 8	219 ± 16	206 ± 32	210 ± 22
P-Mort	2.5%	0.0	0.0	0.0	2.5%	2.9%

Means ± SD.

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; BG = 50-55% purified baker's yeast β-glucans at 108.4 ppm; MOS = 50% purified baker's yeast mannoproteins at 117.3 ppm; BG+MOS = 108.4 ppm BG + 117.3 ppm MOS.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; PI = performance index; P-Mort = phase mortality.

Table 4.3 Effect of yeast cell wall components on broiler performance on day 16 (pre-challenge).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	BG	MOS	BG+MOS
BW (g)	538 ± 40	510 ± 50	531 ± 34	540 ± 35	539 ± 31	549 ± 19
WG (g)	273 ± 27	255 ± 32	271 ± 26	280 ± 27	291 ± 34	292 ± 14
P-F:G	1.44 ± 0.07^{b}	1.44 ± 0.09^{b}	1.37 ± 0.03^{a}	1.37 ± 0.05^{a}	1.36 ± 0.02^{a}	1.34 ± 0.03^{a}
C-F:G	1.32 ± 0.04^{b}	1.32 ± 0.04^{b}	1.29 ± 0.02^{ab}	1.29 ± 0.04^{ab}	1.28 ± 0.02^{a}	1.27 ± 0.02^{a}
FCR	1.21 ± 0.02^{c}	1.20 ± 0.03^{bc}	1.18 ± 0.01^{ab}	1.18 ± 0.02^{ab}	1.17 ± 0.0^{a}	1.17 ± 0.01^{a}
PI	249 ± 32	235 ± 36	257 ± 19	262 ± 21	262 ± 27	262 ± 29
P-Mort	0.0	3.3%	0.0	0.0	0.0	0.0
C-Mort	2.5%	3.3%	0.0	0.0	2.5%	2.9%

[a-c]Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; BG = 50-55% purified baker's yeast β-glucans at 108.4 ppm; MOS = 50% purified baker's yeast mannoproteins at 117.3 ppm; BG+MOS = 108.4 ppm BG + 117.3 ppm MOS.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

The birds in treatments YCW (T3), glucans (T4), mannoproteins (T5), and glucan with mannoproteins (T6) had significantly lower P-F:G (1.37, 1.37, 1.36, and 1.34, respectively) than birds in both control groups, NCh-Cont (T1) and Ch-Cont (T2) (1.44 for both) (Table 4.3). In addition, treatments T5 and T6 had significantly lower C-F:G (1.28 and 1.27) than T1 and T2, but not significantly lower than T3 and T4 (both 1.29). The same scenario was observed in FCR for all treatments.

By day 21 (Table 4.4), post-challenge, the Cp challenge had impacted broiler performance. The average bird weight in the challenge control group was lower than the non-challenge control group by 92 g. The addition of YCW or its components to the broiler diets improved BW ($P = 0.8$). The birds in T6 were higher in BW than birds in T2 by 104 g ($P = 0.075$). WG in T2 (240 g) was significantly lower than in T1 (305 g). Birds in the T6 treatment demonstrated significantly higher WG (306) than the T2 treatment, but not significantly different from other treatments. No significant difference was observed between T3 (285 g), T4 (252 g), and T5 (262), and these treatments were not significantly different from both control groups. No significant differences were observed between treatments in P-F:G, C-F:G, FCR, or PI.

Table 4.4 Effect of yeast cell wall components on broiler performance on day 21 (post-challenge).

	Treatment group[1]					
	NCh-Cont[2]	Ch-Cont[2]	YCW	BG	MOS	BG+MOS
BW (g)[3]	843 ± 67	751 ± 71	816 ± 61	792 ± 75	801 ± 54	855 ± 65
WG (g)	305 ± 31[a]	240 ± 43[b]	285 ± 43[ab]	252 ± 55[ab]	262 ± 44[ab]	306 ± 56[a]
P-F:G	1.46 ± 0.10	1.64 ± 0.21	1.58 ± 0.18	1.73 ± 0.29	1.69 ± 0.19	1.59 ± 0.20
C-F:G	1.38 ± 0.06	1.42 ± 0.07	1.38 ± 0.05	1.41 ± 0.06	1.40 ± 0.03	1.38 ± 0.07
FCR	1.30 ± 0.04	1.33 ± 0.05	1.30 ± 0.04	1.33 ± 0.05	1.31 ± 0.02	1.30 ± 0.07
PI	286 ± 42	246 ± 45	262 ± 51	234 ± 44	225 ± 55	243 ± 50
P-Mort	0.0	0.0	6.7%	12.5%	16.3%	14.3%
C-Mort	2.5%	3.3%	6.7%	12.5%	17.5%	17.1%

[a-c]Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]NCh-Cont = non-challenged control; Ch-Cont = challenged control; YCW = yeast cell wall (YCW) at 250 pm; BG = 50-55% purified baker's yeast β-glucans at 108.4 ppm; MOS = 50% purified baker's yeast mannoproteins at 117.3 ppm; BG+MOS = 108.4 ppm BG + 117.3 ppm MOS.

[2]NCh-Cont and Ch-Cont birds fed control diet, but differ on day 16 and 17 when Ch-Cont birds were challenged with *Clostridium perfringens.*

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

This study was conducted to assess each of the YCW (derived from *Saccharomyces cerevisiae*) components (MOS and glucan) individually or in combination and compare them to the original YCW (the same YCW that was used to extract these components) to eliminate the influence of different sources (Fowler et al., 2015b). The levels of each component were calculated to be equivalent to the content of the corresponding part in the original YCW. The study was conducted in a Cp challenge model introduced on day 16 of age after birds were immunocompromised with 10x the recommended level of bursal disease vaccine (Fowler et al., 2015b; McReynolds et al., 2004).

The results demonstrated no significant difference between treatments in broiler performance on day 10. In previous studies conducted by our lab evaluating baker's yeast cell wall products, no differences were observed in performance, especially when no stress was introduced at this early age. This is in agreement with other findings reported by other studies. M'Sadeq et al. (2015) reported that supplementation of YCW in broiler diets had no significant effect on broiler performance between 0 to 10 days of age.

Administration of bursal disease vaccine 10x the recommended dose has been used to immunocompromised the birds to exaggerate the influence of Cp infection in broilers (McReynolds et al., 2004). The results of the current study demonstrated an improvement in feed utilization on day 16, after being immunocompromised on day 10 and before the Cp challenge. This may suggest that YCW or its components mitigate the adverse effect of the high dose of vaccine.

Following the Cp challenge, on day 21, the non-challenged birds had significantly higher weight gain and improvement in BW compared to the challenged control. This is in agreement with studies described in previous chapters as well as other studies conducted by our lab (Fowler et al., 2015b). Supplementing the starter broiler diets with YCW or its components improved the ability of the birds to tolerate the impact of the Cp challenge. This improvement was significantly higher when the two components of YCW, mannoproteins purified at 50% and glucan at 50-55%, were combined together. The birds in this treatment demonstrated 104 g higher BW than birds in the challenged control group. Previous studies have reported improved broiler performance when fed diets supplemented with YCW after challenge with Cp (Fowler et al., 2015b; M'Sadeq et al., 2015). The results of the current study suggest that both mannoproteins and beta-glucans are equally important, perhaps even synergistic with respect to their effect on broilers undergoing immunogenic stress.

CHAPTER V

PERFORMANCE OF BROILERS FED DIETS SUPPLEMENTED WITH TWO YEAST CELL WALL STRAINS USING TWO FEEDING STRATEGIES

Introduction

In the poultry industry, it is common to reuse old litter for more than one year. However, the old litter provides a suitable environment for different kinds of living organisms, including pathogenic microbes such as coccidia. In addition, increased humidity and pH at warm temperatures enhance the proliferation of pathogenic bacteria in litter. The use of antibiotics at sub-therapeutic levels in animal feed as growth promotors improves animal performance and resistance to pathogens. The application of antimicrobials as growth promoters has been employed in the livestock industry for more than seventy years. However, concerns regarding development of antibiotic-resistant bacteria in animals and potential transfer to humans have introduced the ban of these growth promotors and increased demand for alternatives.

Supplementation of yeast cell wall products in animal feed has been reported as a potential alternative to antimicrobial growth promotors (Fowler et al., 2015a; Fowler et al., 2015b; Hashim et al., 2013; M'Sadeq et al., 2015; Sohail et al., 2012). YCW is capable of changing the microflora community structure in the gastrointestinal tract and changing the histological structure of the gut mucosa (M'Sadeq et al., 2015; Reisinger et al., 2012; Sohail et al., 2012). Pathogenic bacteria that express Type-1 fimbriae are diminished due to the ability of MOS in YCW to link to these types of pathogens such as

Salmonella (Spring et al., 2000). Antimicrobial peptides, important components of the innate immune response, express antimicrobial and immunomodulatory characteristics and are significantly upregulated in the jejunum due to the glucans of YCW (Tian et al., 2016).

Different sources of YCW, such as brewer's and baker's yeast, have been used in the industry. Differences in sources and levels could be one of the reasons different outcomes when YCW is added to animal diet (Fowler et al., 2015a). Studies conducted by our laboratory have demonstrated a quadratic effect of YCW on starter broiler performance under Cp challenge (Fowler et al., 2015b). These studies were conducted using battery cages that are not similar to commercial settings and cannot extend the study beyond 24 days because of size restriction. The purpose of the current study is to evaluate two sources of YCW from two different strains of *Saccharomyces cerevisiae* fed through two different strategies on full-term (1-42 days) broiler performance.

Materials and Methods

Dietary treatments

Two YCW products extracted from two different strains of *Saccharomyces cerevisiae* were used in this study to prepare the dietary treatments (Phileo-Lesaffre Animal Care, Milwaukee, WI). A basal corn/soy based broiler diet (Table 5.1) was formulated during each phase (starter, grower, and finisher) to meet the nutrient requirements suggested by the Ross-308 management handbook. The diet was then divided into five batches that included control (basal diet only, T1); basal diet with 250

ppm of strain 1 YCW in all three phases (T2); basal diet with strain 1 of YCW at 500 ppm in starter phase, 250 ppm in grower phase, and 125 ppm in finisher phase (T3); basal diet with 250 ppm of strain 2 YCW in all three phases (T4); and basal diet with strain 2 of YCW at 500 ppm in the starter phase, 250 ppm in the grower phase, and 125 in the finisher phase (T5).

Animal husbandry

A total of 960 straight-run Ross-308 broiler chicks were randomly assigned to 60 6'x 6' floor pens with 16 birds/pen in a randomized block design with 12 pen replicates per treatment. The birds were placed in floor pens on used pine-shaving litter. The study utilized a full-term 42-d, 3-phase rearing program with a 21-d starter, 14-d grower, and 7-d finisher phase. Mortality within the first three days was replaced with new birds that had been fed the control diet. Feed and water were provided ad libitum. Body weight (BW) and feed intake were collected for each phase, and mortality was monitored daily. The study was conducted at the Poultry Science Research Center and was approved by the Texas A&M Institutional Animal Care and Use Committee.

Statistical analysis

The experiment was designed as a randomized block. Data were analyzed as a one-way ANOVA using the GLM procedure of SPSS. Means deemed significant at $p \leq 0.05$ were separated using a protected Duncan's Multiple Range Test.

Table 5.1 Composition and nutrient content of the experimental basal diets.

Ingredient	Starter	Grower	Finisher
Corn (%)	61.6	66.57	70.45
Dehulled soybean meal	31.7	27.20	22.84
Dl-Methionine 98% (%)	0.23	0.24	0.11
Lysine HCl (%)	0.19	0.16	0.13
Soybean oil (%)	2.20	2.21	3.15
Limestone (%)	1.44	1.48	1.35
Mono-dicalcium	1.55	1.39	1.38
Salt (%)	0.51	0.41	0.14
Trace minerals premix[1]	0.05	0.05	0.05
Vitamin premix[2] (%)	0.25	0.25	0.25
Coban 90	0.05	0.05	-
Sodium bicarbonate	-	-	0.17
Calculated nutrient content			
CP (%)	22.00	20.00	18.00
ME (Kcal/kg)	3050	3100	3200
Crude fat (%)	3.94	4.04	4.99
Crude fiber (%)	2.14	2.02	1.90
Calcium (%)	0.95	0.92	0.85
Available phosphate (%)	0.45	0.41	0.40
Sodium (%)	0.22	0.18	0.12
Methionine (%)	0.56	0.54	0.39
Lysine (%)	1.31	1.15	1.00

[1]Trace minerals premix added at this rate yields (mg/kg): zinc, 60.0; manganese, 60.0.

[2]Vitamin premix added at this rate yields (per kg): vitamin A, 11 kIU; vitamin D_3, 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; B_{12}, 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; B_6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

Results and Discussion

This study was conducted to assess the influence of supplementation with two YCW products from two different *Saccharomyces cerevisiae* strains. YCW supplementation was conducted in two different inclusion methods: supplementing diet with YCW at a consistent level, 250 ppm, during three rearing phases, or adding YCW to the diet at three levels starting with 500 ppm in the starter phase, 250 ppm in the grower phase, and 125 ppm in the finisher phase. The birds were placed in floor pens on used pine-shaving litter to mimic industry-type rearing conditions.

The results of starter phase are demonstrated in table 5.2. At the end of the 21-day starter phase, birds that were fed diets supplemented with any of the YCW products at any of level performed better than the control birds. There was a 6.6 – 9.6% increase in BW for all of the YCW birds over the control birds. The BW of birds in T2, T3, T4, and T5 was significantly higher than BW of birds in the control group (819, 830, 807, 812, and 757 g, respectively). The YCW treatments improved WG with 7.3 – 10.2% more gain than control birds. The WG of broilers in T2, T3, T4, and T5 treatments was significantly higher than the WG of broilers in the control (T1) group (776, 786, 765, 768, and 713 g, respectively). There were no significant differences in P-F:G, C-F:G, FCR, or PI at day 21 of age.

Table 5.2 Effect of two different strains of yeast cell wall on broiler performance on day 21 (Starter Phase).

	Treatment group[1]				
	T1	T2	T3	T4	T5
BW (g)	757 ± 49[b]	819 ± 54[a]	830 ± 51[a]	807 ± 38[a]	812 ± 39[a]
WG (g)	713 ± 49[b]	776 ± 53[a]	786 ± 50[a]	765 ± 38[a]	768 ± 38[a]
P-F:G	1.37 ± 0.17	1.37 ± 0.02	1.35 ± 0.03	1.35 ± 0.02	1.36 ± 0.02
FCR	1.29 ± 0.15	1.30 ± 0.02	1.28 ± 0.02	1.28 ± 0.02	1.29 ± 0.02
PI	266 ± 40	285 ± 21	292 ± 23	281 ± 17	284 ± 16
P-Mort	0.52%	0.0	0.52%	1.62%	0.0

[a-b]Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]T1 = control; T2 = strain 1 yeast cell wall (YCW) at 250 pm all phases; T3 = strain 1 YCW at 500, 250, & 125 ppm starter, grower, & finisher phases respectively; T4 = strain 2 YCW at 250 ppm all phases; T5 = strain 2 YCW at 500, 250, & 125 ppm starter, grower, & finisher phases respectively.

P-F:G = phase feed:gain; PI = performance index; P-Mort = phase mortality.

At the end of the grower phase (Table 5.3), on day 35, the trends of improved BW and WG were similar to the starter phase, and additional improvement was noticed in other performance variables such as P-F:G, C-F:G, FCR, and PI. At that age, all birds except control birds were fed diets supplemented with the same inclusion rate of YCW at 250 ppm. The supplementation of broiler diets with any of the YCW products significantly improved BW. The increase in average BW for phase 2 was 5.4 – 7.8% more in YCW treatments than with control. The average BW of the birds in T1 was 2140 g, T2 was 2284 g, T3 was 2307 g, T4 was 2296 g, and T5 was 2256 g. The WG was significantly higher in T2, T3, T4, and T5 than control (1465, 1478, 1489, 1445, and 1384 g, respectively). Birds in T4 demonstrated significant improvement in P-F:G (1.55) and FCR (1.45), and C-F:G (1.48) was numerically lower (P = 0.057) than all other treatments. Performance index was significantly improved in all YCW treatments (409-422) compared to control (389).

At the end of the finisher phase (Table 5.4), at 41-days-old, birds in any of the YCW treatments had significantly higher BW and PI than control birds. The average BW in control birds was 2875 g, significantly lower than the average BW of T2, T3, T4, and T5 birds (3032, 3073, 3045, and 3013 g, respectively). The PI of the control group (415) was significantly lower than the PI values of T2, T3, T4, and T5 (440, 447, 442, and 433, respectively). There were no significant differences between treatments in WG, P-F:G, C-F:G, or FCR.

Table 5.3 Effect of two different strains of yeast cell wall on broiler performance on day 35 (Grower phase).

	Treatment group[1]				
	T1	T2	T3	T4	T5
BW (g)	2140 ± 81[b]	2284 ± 102[a]	2307 ± 95[a]	2296 ± 85[a]	2256 ± 88[a]
WG (g)	1384 ± 55[b]	1465 ± 63[a]	1478 ± 67[a]	1489 ± 60[a]	1445 ± 68[a]
P-F:G	1.59 ± 0.05[b]	1.59 ± 0.03[b]	1.59 ± 0.03[b]	1.55 ± 0.02[a]	1.60 ± 0.03[b]
C-F:G[2]	1.52 ± 0.06[b]	1.51 ± 0.02[b]	1.50 ± 0.02[b]	1.48 ± 0.01[a]	1.52 ± 0.02[b]
FCR	1.49 ± 0.06[b]	1.49 ± 0.02[b]	1.48 ± 0.02[b]	1.45 ± 0.01[a]	1.49 ± 0.02[b]
PI	389 ± 25[b]	419 ± 21[a]	422 ± 23[a]	422 ± 21[a]	409 ± 20[a]
P-Mort	0.52%	0.0	0.52%	0.52%	1.0%
C-Mort	1.0%	0.0	1.0%	2.1%	1.0%

[a-b]Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]T1 = control; T2 = strain 1 yeast cell wall (YCW) at 250 pm all phases; T3 = strain 1 YCW at 500, 250, & 125 ppm starter, grower, & finisher phases respectively; T4 = strain 2 YCW at 250 ppm all phases; T5 = strain 2 YCW at 500, 250, & 125 ppm starter, grower, & finisher phases respectively.

[2]P-value = 0.057.

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

Table 5.4 Effect of two different strains of yeast cell wall on broiler performance on day 42 (Finisher phase).

	Treatment group[1]				
	T1	T2	T3	T4	T5
BW (g)	2875 ± 75[b]	3032 ± 110[a]	3073 ± 126[a]	3045 ± 91[a]	3013 ± 103[a]
WG (g)	733 ± 39	745 ± 34	763 ± 45	747 ± 39	754 ± 28
P-F:G	1.98 ± 0.11	2.00 ± 0.08	1.97 ± 0.05	1.99 ± 0.07	1.97 ± 0.06
C-F:G	1.63 ± 0.05	1.63 ± 0.02	1.62 ± 0.02	1.61 ± 0.02	1.63 ± 0.02
FCR	1.61 ± 0.05	1.61 ± 0.02	1.60 ± 0.02	1.58 ± 0.02	1.61 ± 0.02
PI	415 ± 17[b]	440 ± 20[a]	447 ± 24[a]	442 ± 15[a]	433 ± 19[a]
P-Mort	0.0	0.5%	0.0	0.0	0.52%
C-Mort	1.0%	0.5%	1.0%	2.1%	1.6%

[a-b]Means ± SD within a row lacking a common superscript differ ($P \leq 0.05$).

[1]T1 = control; T2 = strain 1 yeast cell wall (YCW) at 250 pm all phases; T3 = strain 1 YCW at 500, 250, & 125 ppm starter, grower, & finisher phases respectively; T4 = strain 2 YCW at 250 ppm all phases; T5 = strain 2 YCW at 500, 250, & 125 ppm starter, grower, & finisher phases respectively.

P-F:G = phase feed:gain; C-F:G = cumulative feed:gain; PI = performance index; P-Mort = phase mortality; C-Mort = Cumulative mortality.

The results of this study demonstrated improved performance in broilers reared on a reused pine shavings floor and fed diets supplemented with any of the YCW products using any of the two supplementation programs. It was reported in previous studies that doses of YCW have a quadratic effect on starter broiler BW subjected to Cp challenge (Fowler et al., 2015b). The optimum YCW inclusion rate was calculated to be 295 ppm. Fowler et al. (2015a) reported no significant difference in broiler performance between control corn/soybean diet and YCW treatments at 250 ppm during the starter phase when birds were raised on fresh pine shavings. However, this study reported a significantly higher WG in birds fed YCW at 250 ppm than control birds during the grower phase. It was also reported that YCW derived from *Saccharomyces cerevisiae* had no significant effect on broiler performance at 24 or 35 days old compared to control when new hard wood litter was used to cover the floor pens (M'Sadeq et al., 2015). In this study, the YCW was added to the diets at 800, 400, 200 ppm during the starter, grower, and finisher phases, respectively. Nevertheless, YCW did improve BW on day 24 and 35 when birds were challenge with attenuated *Eimeria spp* oocysts on day 9 and Cp on days 14 and 15. The findings of the current study suggest that YCW can improve broiler performance when raised on reused floor beds that can introduce different pathogenic challenges.

CHAPTER VI

SUMMARY

The latest joint report from the Centers of Diseases Control and Prevention and the U.S. Department of Health and Human Services reported that improperly prepared chicken meat is the second-greatest source of foodborne illness outbreaks after fish in the United States. The use of antibiotic growth promotors (AGPs) in poultry feed since the 1940's has not only improved the performance of the birds, but has also controlled the virulence of several pathogenic challenges at the farm level which undoubtedly has to some extent helped to limit foodborne illnesses as a result of poultry consumption. One of the pathogenic challenges that can be somewhat controlled by AGPs is necrotic enteritis (NE) caused by *Clostridium perfringens* (Cp). The deleterious outcomes of NE have dramatic financial impacts on the poultry industry because of the chronic negative influence on bird performance. This impact on performance is a consequence of poor digestion and absorption of the nutrients and thus low FCR in the long term.

There is growing concern with the employment of antibiotics at sub-therapeutic levels in animal feed due to reports of transferable antibiotic resistant bacteria from food animals to humans. This concern is amplified with medically important growth promotors. In addition, the FDA has issued a Guidance for Industry and proposed a Veterinary Feed Directive that will take effect at the end of 2016. These two documents restrict poultry and livestock producers from non-medical application of antimicrobials that are considered medically important to humans. In addition, the recent report of the

National Action Plan for Combatting Antibiotic Resistant Bacteria indicated that one of their goals to slowing the emergence of antibiotic resistance and preventing its spread is by ceasing the use of medically important antibiotics in food animal industry at sub-therapeutic levels. These challenges have increased the efforts to invent and/or develop new antibiotic alternatives that are capable of improving or at least maintaining performance of livestock animals while at the same time boosting their resistance to pathogenic challenges.

Mannan oligosaccharides and β-glucan are major components of the cell wall of many living organisms such as yeast and algae. Macrophages and other phagocytes express different pathogen recognition receptors (PRRs) such as glucan and mannose receptors. Those PRRs recognize specific structures known as MAMPs or PAMPs that are found on several microorganisms but not on host cells. Therefore, YCW, which is a rich source of mannan and β-glucans, has demonstrated promising positive influence on animal performance and health.

The literature has reported contradictory results from the use of YCW, MOS, and β-glucan. These conflicts in reported outcomes could be the result of using YCW preparations that are not purely MOS or glucans and assuming that only one of those components is the main influencer. In addition, different purification levels can be found for YCWs in general or their components. Moreover, the MOS and glucans can be derived from many sources beside yeast such as algae, mushroom, and gum.

For this dissertation, the purification level of YCW preparations demonstrated significant impact on broiler performance with the more highly purified YCW product

able to alleviate the impact of Cp on challenge birds. In addition, the metabolic pathways in birds challenged with Cp and fed purified YCW were more likely to be similar to the metabolic pathways of non-challenged birds.

The highly purified β-glucan derived from *Euglena gracilis* demonstrated significant influence on broiler performance when birds were challenged with Cp. It was noticed that addition of this product at levels no greater than 30 ppm can be sufficient to achieve maximum performance in starter broilers subjected to Cp challenge. However, highly purified MOS derived from *Saccharomyces cerevisiae* did not significantly improve performance of Cp challenged starter broilers.

In the third study, chapter 4, Both MOS and β-glucan preparations were extracted from the YCW of *Saccharomyces cerevisiae* and purified to 50 – 55%. Neither one of these preparation demonstrated significant effect on Cp challenged starter broilers. However, addition of both preparations to the starter broiler diets at levels similar to their proportions in commercial YCW product improved broiler performance. This may indicate that these two components of YCW have a synergistic effect.

The inclusion of any of the two different products of YCW derived from two different strains of Saccharomyces cerevisiae in full-term broiler diets either at 250 ppm continuously, or starting with 500, 250 and then 125 ppm during the finisher phase demonstrated similar influence on broiler performance. However, adding 250 ppm throughout the rearing period could be more economically feasible since less of the YCW would be included.

In conclusion, YCW purification plays important role in improving broiler performance response to this supplement. The components of YCW, MOS and β-glucan, appear to work more effectively when combined. The β-glucan derived from algae, Euglena gracilis, demonstrated significant outcomes in starter broiler performance that was not reported in yeast β-glucan. This could be as a result of the structure of algae glucan used in this study that was unbranched 1,3-β glucan.

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