PREBIOTIC PROPERTIES OF YEAST CELL WALL MANNANOLIGOSACCHARIDES AND GUAR GUM GALACTOMANNANS IN STARTING BROILERS

A Dissertation

by

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ABSTRACT

Two studies were designed to evaluate the growth promoting and prebiotic properties of Yeast Cell Wall (YCW) containing mannanoligosaccharides (MOS) and guar gum galactomannans in starting broilers. In study one, the effects of different sources and concentrations of YCW-MOS and a blend from both the sources were investigated in starting broilers under both challenged (immune stress and *Clostridium perfringens* challenge) and unchallenged conditions through a series of 6 challenged and 4 unchallenged experiments. Weekly body weights, feed consumption, and daily mortality were recorded. Each experiment was terminated after 3 weeks. YCW-MOS had no effect in the unchallenged birds. Pooled data analysis of challenged broilers revealed no effect of source of YCW-MOS. Both the products tested produced significant improvement in growth rate compare to the control birds. However, the blend of YCW-MOS showed approximately 15% improvement in growth rate with 10% reduction in feed conversion rate (FCR). The optimum dose of tested YCW-MOS products in starting broilers is determined to be 250 ppm. YCW-MOS additives produced increased body weight with a reduction in FCR and may be considered as alternatives to antibiotic growth promoters.

In study two, newly hatched broiler chicks (24 pens, 6 replicates per treatment) were randomly distributed among four dietary treatments to evaluate the effects of guar gum galactomannans (GG) with and without Mannanase Guar® enzyme in starting broilers. Effects of dietary treatments (negative control, positive control-YCW product

Safmannan (YCW-S) at 500 ppm, GG at 500 ppm and GG at 500 ppm with enzyme (GGE) on growth, FCR, apparent ileal energy digestibility (AIED), intestinal histomorphology and microbial ecology were investigated. No significant differences were observed in body weight, feed conversion, mortality and productivity index. GG diets produced significantly reduced AIED, villus height, and increased crypt depth compared to the control. Broilers receiving GGE had overall intestinal villus height and AIED equal to YCW-S. Microbial patterns from the YCW-S and GGE treated broilers grouped together with a 95.6% similarity coefficient suggesting near identical microbial populations between these two groups. GG may have potential to consider as a prebiotic in starting broilers when used with an appropriate exogenous enzyme.

DEDICATION

To my family.

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NOMENCLATURE

YCW	Yeast cell wall
MOS	Mannanoligosaccharides
FOS	Fructooligosaccharides
GG	Guar gum galactomannans
GGE	GG with Mannanase Guar® enzyme
BW	Body weight per bird
WG	Weight gain per bird
FC	Total feed consumption
FCR	Feed conversion ratio
PI	Productivity index
MORT	Mortality rate (%)
NE	Necrotic enteritis
NEL	Necrotic enteritis lesion score
AIED	Apparent ileal energy digestibility
IBD	Infectious bursal disease
S	Safmannan®
P	Pronady®
FR	France
BR	Brazil
CR	Cedar Rapids

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CHAPTER I

INTRODUCTION

Antibiotics have been in use for centuries in one form or another. The previous century witnessed a dramatic improvement in human health with the development of antimicrobial drugs. Although most of these drugs are used to treat diseases in humans and animals, it is not uncommon to add antibiotics at very low levels to the animal feeds to improve growth rates. During the 1950's, initial results with supplemental antibiotics suggested an overall improvement of 20-25% growth rate in poultry (Leeson and Summers, 2001). Antibiotic feed additives may influence performance by reducing the negative effects of highly variable disease conditions. Over the decades, several antibiotic resistant bacteria emerged posing a potential public health threat to humans. Emergence of resistant bacteria has been linked to the excessive use of sub-therapeutic doses of antimicrobial growth promoters. Based on several investigations, the European Union restricted the use of antibiotic growth promoters in animal husbandry since January 1, 2006. There is increasing public pressure to limit or withdraw the use of antimicrobial feed additives in the USA. Under such circumstances, to meet the food needs of increasing world population, animal protein production needs to be substantially increased. The pressing needs to be on developing potential alternatives for antibiotics in animal feeds. In the past two decades, several investigators evaluated the use of probiotics, prebiotics, synbiotics and organic acids as antibiotic alternatives in the animal agriculture.

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the activity of one or more bacteria leading to better host health (Gibson and Roberfoid, 1995). Therefore, the primary characteristic of a prebiotic is to provide a substrate for beneficial gut microflora. Many of the non-digestible carbohydrates such as mannanoligosaccharides (MOS), fructooligosaccharides (FOS), and galactomannans have been investigated for prebiotic functions. Cereal grains are the primary energy source in animal feeds and contain about 80% carbohydrates (both starch and non-starch polysaccharides). All sources of potential energy are not completely digested by chickens. Monogastric animals lack enzymes to digest non-starch polysaccharides (NSP's). The NSP's make up to 10-30% of the carbohydrates present in cereals. Oligosaccharides and NSP's are increasingly being investigated for this prebiotic activity. MOS and FOS inclusion at certain concentrations in poultry diets may improve performance, increase colonization of beneficial bacteria and reduce pathogenic bacteria. Yeast cell wall (YCW) derivatives consisting MOS are known to modulate immune response, and to influence intestinal microflora, thereby improving animal health under stress conditions. Research findings are suggesting that dietary inclusion of YCW in poultry diets may result in improved performance when subjected to immune stress or challenged with pathogens (*Salmonella* or *C.perfringens*).

Galactomannan, a non-starch polysaccharide naturally occurring in several plant legumes, is known to depress nutrient utilization by increasing the viscosity of intestinal contents. Adding exogenous enzymes to reduce the negative effects of some NSP's is a common practice in poultry feeding. These enzymes improve digestibility of the

polysaccharides otherwise not digested by the host system. Galactomannan gum obtained from the plant legume guar may be considered for prebiotic functions. Conflicting results were reported on the effects of residual guar gum on layer and broiler performance. Guar gum galactomannan has a hypo-cholesteremic effect in rats and humans.

Both oligosaccharides and non-starch polysaccharides have been proposed as prebiotic compounds to replace antimicrobial feed additives in animal husbandry. In this context, the objectives of this research are: 1) to investigate the effects of different sources and doses of YCW-MOS products on growth rate and feed conversion ratio in starting broilers; 2) to determine the best MOS-YCW product or combination of products among those; 3) to determine the optimal concentration or dose of YCW-MOS in starting broilers and 4) to investigate the effects of guar gum galactomannans with and without Mannanase Guar® on starting broiler performance, apparent ileal energy digestibility, intestinal histomorphology and microbial ecology.

CHAPTER II

LITERATURE REVIEW

ANTIBIOTIC GROWTH PROMOTERS AND ANTIBIOTIC RESISTANCE

Antibiotic growth promoters (AGPs) have been used extensively in animal feeding over the last few decades to increase growth rate and inhibit potential intestinal pathogens. The use of in-feed antibiotics is not without any risks or limitations. Sub therapeutic use of antibiotics in animal feeds resulted in emergence of resistant bacteria, which is a major problem of public health interest. Animals and humans share some common bacterial pathogens of public health interest. Widespread use of antibiotics in animals and humans plays a significant role in the emergence of antimicrobial drug resistant bacteria (Conly, 2002). There is well documented evidence (Bates et al, 1994; Coque et al, 1996; Van den Bogaard et al, 1997; Aarestrup et al, 2000; Van den Bogaard et al, 2000; Wegener, 2003; Gupta et al, 2004; Silbergeld et al, 2008) in the literature describing the link between excessive use of antimicrobials in animal agriculture and development of antimicrobial resistant bacteria of human interest.

Bacteria are in a sense biochemical factories that respond to antibiotics with metabolic changes in an attempt to counter them. Bacteria use a kind of trial and error mechanism to create chemical responses to antibiotics. Once the right biochemical combination to resist the antibiotic in question develops, the new mutated strain will flourish. Generally it will take bacterial generations to develop resistance. In any animal system, when encountered with antibiotics, bacteria try to develop one or another mechanism to resist the action of the antibiotic. Bacterial resistance depends on different

mechanisms. Bacteria may be inherently resistant to an antibiotic (Wright, 2005; Yoneyama and Katsumata, 2006). Bacteria may exhibit resistance to the antibiotic by preventing antimicrobial access to their targets (Wright, 2005; Yoneyama and Katsumata, 2006), or by altering the target sites of antimicrobials (Rachakonda and Cartee, 2004), or by enzyme inactivation, which selectively target and inactivate the antibiotic (Yoneyama and Katsumata, 2006).

Silbergeld et al, 2008 summarized the importance of agricultural antimicrobial drug use as a major driver of emerging antimicrobial resistance throughout the world for the following reasons - "It is the largest use of antimicrobials worldwide; much of the use of antimicrobials in agriculture results in sub-therapeutic exposures of bacteria; drugs of every important clinical class are utilized in agriculture; and human populations are exposed to antimicrobial-resistant pathogens via consumption of animal products as well as through widespread release into the environment".

Antibiotics are used in animal agriculture for therapeutic purposes, prophylactic purposes and also as growth promoters. The majority of the antibiotics are administered to animals with their feed or water as it is a practical way of giving medicines to large groups of animals. One obvious disadvantage associated with this process is that the sick, weaker animals with appetite loss consume smaller amounts of antibiotics than healthy animals. Antibiotic feed additives or growth promoters are given at sub-therapeutic concentrations. In such cases there is a chance that bacteria become resistant to that particular antibiotic (Wegener, 2003) and later the resistant strains propagate.

The evolution of glycopeptide resistant enterococci (GRE) could be associated with the use of the avoparcin, glycopeptide antibiotic, as a growth promoter in food animals (Bates et al, 1994). This particular antibiotic (avoparcin) has never been approved for use in the USA but was fairly common in Australia and the European Union. Introduction of vancomycin and pristinamycin in swine production was associated with increased prevalence of resistant enterococci from human fecal samples in the Netherlands (Van den Bogaard et al, 2000). Several investigations on the development of resistant enterococci isolated from animal faeces and from food of animal origin, in multiple countries, confirmed the relationship between the use of antimicrobial growth promoters and high levels of resistance in enterococci (Coque et al, 1996; Aarestrup et al, 2000; Van den Bogaard et al, 2002).

Prophylactic treatment of poultry with fluoroquinolones resulted in increasing prevalence of ciprofloxacin-resistant *Campylobacter* species in the United States (Gupta et al, 2004). Their results proved that the source of fluoroquinolone-resistant *Campylobacter* infections was the consumption of poultry colonized with resistant strains rather than selection for *Campylobacter* in the human gut after clinical fluoroquinolone use to treat illness. Developing an animal reservoir of antimicrobial resistant bacteria is the major factor behind transmission of resistance to humans (Threlfall et al, 2000). Threlfall et al, (2000) also indicated that no resistance was observed in *C. jejuni* isolates tested from the poultry that had been treated therapeutically with enrofloxacin. These findings suggest that sub-therapeutic use of antimicrobials is the major reason in developing resistance. Unicomb et al, (2006)

reported that the relatively low rate of fluoroquinolone resistance in clinical isolates in Australia has been attributed to the fact that this drug was never used in animal agriculture. Use of virginiamycin as a growth promoter was linked to the carriage of Quinupristin-Dalfopristin-resistant enterococci in healthy humans (Van den Bogaard et al, 1997).

Key determinants in transmission of the resistant bacteria to humans are rate of spread of resistant bacteria from animals to the environment, and rate of spread in the food production chain (Wegener, 2003). Bacteria from animals spread to the food products during slaughter and processing. Direct transmission of resistant enterococci between animals and farm workers has been reported by Van den Bogaard et al, 2002. A major determinant of developing resistance appears to be sub therapeutic antimicrobial doses.

Based on several documentations that state that the use of antibiotic growth promoters in animal agriculture has led to the creation of a major food-animal reservoir of resistant bacteria, more importantly, further spreading of the resistant bacteria to humans by animal contact, food, or the environment, the European Union imposed a ban on all the Antibiotic Growth Promoters (since January 1, 2006) that belong to classes also used in human medicine (Wegener, 2003). In 2004, FDA approved withdrawal of the new animal drug application enrofloxacin for prophylaxis or growth promotion in poultry (Davidson, 2004). This was a major decision by the FDA and was the first occasion that a previously approved antimicrobial agent was removed from the U.S. market because of concerns about antimicrobial drug resistance. Although there are

some safety concerns, antimicrobial growth promoters are still used in animal feed in the United States.

The World Health Organization recommends that antibiotic growth promoters should be prohibited in animal feeds. So, indiscriminate use of antibiotic growth promoters in animal production has been questioned because of the potential associated problems. The European Union prohibited the use of antibiotic growth promoters in animal industry since January 1, 2006 and there may likely be a ban on the use of growth stimulating antimicrobial agents in the United States in the near future. All of these concerns related with the therapeutic use of antibiotics in food producing animals stimulated the scientific community as well as producers to identify alternatives to sub-therapeutic antibiotic use in the animal feeding. Some alternatives to antibiotics are probiotics, prebiotics, organic acids and various plant extracts (Griggs et al, 2005).

PREBIOTICS AND YEAST CELL WALL PRODUCTS IN POULTRY

Antibiotic growth promoters have made major contributions to the profitability of animal agriculture. With increasing pressure to limit or withdraw AGPs from the feed, the incidence of intestinal disease may increase in the future. Researchers are looking for ways to enhance gut health as maintaining good gut health is critical for growth and productive performance of animal when no antibiotics are added to feed. Any of the non-antibiotic growth promoters so far suggested in the literature cannot compensate completely for the absence of antimicrobial feed additives in the animal husbandry. A good alternative must not only improve the performance of the birds but also be

economical to add. For the past decade, extensive research has been conducted over the dietary supplementation of probiotics and prebiotics in poultry production.

The concept of prebiotics was first introduced by Gibson and Roberfroid in 1995. They defined prebiotics as "a food ingredient that affects the functions of the body in a targeted manner so as to exert positive effects that may, in due course, justify health claims". Generally prebiotics are non-digestible carbohydrates with beneficial effects on host health by selective stimulation of one or more bacteria in the GI tract (Gibson and Roberfroid, 1995). Oligosaccharides such as fructooligosaccharides (FOS) and mannanoligosaccharides (MOS) are the common prebiotic compounds extensively investigated as AGP alternatives in animal production (Ammerman et al, 1989; Kumprecht et al, 1997; Fukata et al, 1999; Spring et al, 2000).

The available literature on the efficacy of oligosaccharide prebiotics in poultry feeding offers conflicting results. No difference in the performance was observed when turkeys were fed with different amounts of a commercial FOS preparation (Raftilose P95, Orafti, Belgium), containing 95% oligofructose (Juskiewicz et al, 2006), different concentrations of inulin (FrutafitInulin Tex, Holland) and a commercial MOS product (Bio-Mos®, Alltech Inc., Nicholasville, KY) (Stanczuk et al, 2005), whereas Sims et al, 2004 reported an improved live weight in turkeys when fed with Bio-Mos® (Alltech Inc., Nicholasville, KY) supplemented diets.

FOS inclusion in broiler diets has been demonstrated to decrease the levels of pathogenic bacteria such as *E. coli* and *Salmonella* and to enhance the levels of beneficial bacteria such as *Lactobacillus* and *Bifidobacteria* (Fakuta et al, 1999; Xu et al,

2003). Dose dependent effects of FOS (Meioligo-P®, Meiji Seika Kaisha Ltd., Tokyo, Japan) on average daily weight gain and feed conversion ratios in male broilers were reported by Xu et al. 2003.

The addition of YCW fractions in animal feeding has been extensively investigated for the past decade. Dietary inclusion of YCW, which has been derived from *Saccharomyces cervisiae*, in animal feeds resulted in improved performance in broilers (Spring et al, 2000; Baurhoo et al, 2007). The cell wall determines the shape and integrity of the yeast. The YCW consists of two layers – the inner layer is made of β-1,3- and β-1,6-glucans that is complexed with chitin and the outer layer is made up of mannoproteins (Osumi 1998) (Figure 2-1). The majority of the mannoproteins are covalently linked to the inner glucan layer and so referred to as the mannoprotein complex. Cell walls represent 26-32% of the dry weight of the cell in yeasts. Mannoproteins constitutes 40% of the cell dry mass, and are the major source of MOS in YCW, whereas beta-glucans account for 60% of the cell wall dry mass. Variation in the YCW composition was reported based on the strain origin and the commercial process applied to get the product (Aguilar-Uscanga and Francois, 2003). Therefore, the efficiency of YCW-MOS as feed additives may differ depending on the source to improve chicken performance. Beta-glucans, which are part of YCW, have a variety of biological properties, and are considered as immune modulator substances (Miura et al, 1996). Improved humoral immune responses were observed in birds fed with MOS YCW (Cotter, 1997; Ghosh et al, 2012).

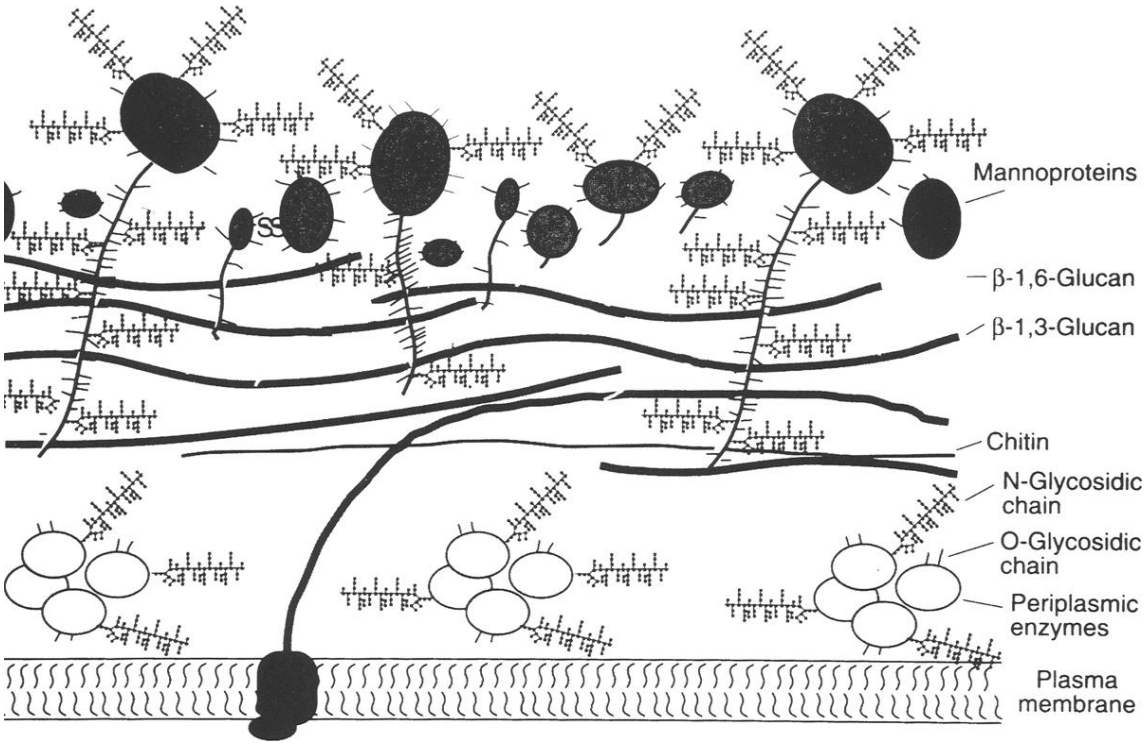

Figure 2-1: Structure and composition of yeast cell wall-mannoproteins are the primary source of the mannanoligosaccharides (Osumi 1998)

Bio-Mos® has been shown to decrease the prevalence of *Salmonella*, expressing type-1 fimbriae, in young broilers (Spring et al, 2000), to increase the intestinal villus height and counts of beneficial bacteria (Baurhoo et al, 2007). The increased villus height offers a larger surface for nutrient absorption and thereby is associated with increased growth rates. A meta-analysis study by Hooge, (2004) showed that dietary MOS may reduce the severity of coccidiosis infection in broilers. This analysis also reported an improved body weight, feed conversion ratio and decreased mortality. In addition, Bio-Mos® supplementation has been demonstrated to improve intestinal health benefits versus antibiotics (as shown with villus height and goblet cell number), to increase colonization of beneficial bacteria and to decrease pathogenic bacteria (Baurhoo et al, 2009), and to alter intestinal microbiota (Geier et al, 2009). Thus, MOS improves the structural integrity of the small intestine. Bio-Mos® and FOS (Fibrulose-F97, Cosucra Group Warcoing, Warcoing, Belgium), however, did not influence the performance of broilers under normal conditions (Baurhoo et al, 2009; Geier et al, 2009).

The gastro intestinal tract harbors a variety of microflora, consisting of both pathogenic and beneficial microbes. Pathogenic bacteria must adhere to the mucosal surfaces of the intestine for successful colonization. Targeting the bacteria attachment sites is an important strategy in reducing the pathogenic bacteria counts. Intestinal pathogens such as *Salmonella* and *E. coli* contain mannose specific type-1 fimbriae (adhesion organelles, which facilitate adherence to mucosal surface). Mannans in YCW act as high affinity ligand for bacteria. So, bacteria with mannose specific type-1

fimbriae bind to the MOS instead of binding to intestinal epithelium (Newman 1994). YCW-MOS serves as an alternate binding site for the bacteria and this MOS-bacteria complex can pass undigested through the gut (Spring et al, 2000). Reduced colonization of *Salmonella* and *E. coli* (Spring et al, 2000), *E.*coli and *Campylobacter* (Baurhoo et al, 2009) were reported when Bio-Mos® was added to chicken diets. Morales-Lopez et al, (2010) suggested that the addition of YCW to the diets enhanced gut maturation by increasing the mucosal resistance to microbial translocation.

There is an added advantage of using prebiotics in place of antimicrobial feed additives as prebiotics do not have any known side effects on the host system. Antibiotics not only kill the pathogenic bacteria but also eliminate beneficial bacteria, which are essential for maintaining good gut health. Overall, advantages of dietary supplementation of YCW-MOS include significant increase in weight gain and feed conversion; enhanced intestinal function, decreased mortality, improved colonization of beneficial bacteria and reduced counts of pathogenic bacteria. (Spring et al, 2000; Xu et al, 2003; Chen et al, 2007; Benites et al, 2008; Baurhoo et al 2009; Geier et al, 2009; Ghosh et al, 2012).

SIGNIFICANCE OF NECROTIC ENTERITIS

The general health condition of the animal determines its performance, which in turn depends on several factors such as management, environment, nutrition, genetic potentiality, exposure to microbes etc. Health and nutrition are obviously interdependent. Maintaining good gut health is extremely important for wellbeing and productivity of the animal. A favorable intestinal environment coupled with the high availability of

nutrients are the key factors that influence the incidence of enteric pathogens such as *C. perfringens*. Economic losses associated with enteric pathogens have become a serious issue in the poultry industry as the use of controversial antibiotic growth promoters has declined resulting in increased prevalence of intestinal pathogens (Van Immerseel et al, 2009).

Necrotic Enteritis (NE) is an acute enteric disease associated with the gram positive, spore-forming anaerobic bacteria *Clostridium perfringens.* It is a widespread disease in broilers causing significant global economic losses to the poultry industry. Van der Sluis, (2000) estimated a total global financial loss of over 2 billion annually as a result of NE. Several predisposing factors like diet composition, exposure to stress, and the presence of coccidiosis contribute to the occurrence of NE in broilers. Diets rich in indigestible non-starch polysaccharides (wheat, barley, and oats) are known to increase intestinal viscosity and reduce nutrient digestibility (Branton et al, 1987; Craven 2000; Kocher, 2003) predisposing broilers to NE.

NE in poultry was first described by Parish (1961). The infection may present as an acute clinical form characterized by decreased appetite, depression, diarrhea and necrosis of the intestines thus resulting in increased mortality (Ficken and Wages, 1997) or as a sub-clinical form causing damage to the intestinal mucosa leading to reduced nutrient absorption, decreased weight gain and impaired feed efficiency (Stutz and Lawton, 1984; Hofacre et al, 2003). The sub-clinical form of NE has become more prevalent in recent years where in no clinical symptoms are observed, but damaged intestinal mucosa causes production losses.

Controlling the incidence of NE is an important issue for the commercial poultry industry. Over the past several decades, sub-therapeutic use of antimicrobial feed additives has helped control the prevalence of NE. Without the use of antimicrobial feed additives, the incidence of NE is a major production concern (Kaldhusdal and Lovland, 2000). Foodborne disease outbreaks caused by *C. perfringens* can often be traced back to poultry (Hook et al, 1996) making it not only an economically important disease, but also of potential public health threat.

GUAR GUM GALACTOMANNANS AND EXOGENOUS ENZYMES

Carbohydrates in poultry feeds

Carbohydrates are organic compounds which can be major sources of energy for poultry. All sources of potential energy are not completely digested by chickens. The amount of energy available to the bird is a deciding factor that determines growth rate and productive performance. Energy availability, in turn, depends on the digestibility of carbohydrates in GI tract. Complex carbohydrates are broken down into simple monosaccharides and then absorbed into the system to provide energy. Some carbohydrates are indigestible by gastric enzyme systems in chickens. The major factor influencing carbohydrate digestion is the content of indigestible polysaccharides in the diet. These indigestible polysaccharides are collectively called Non-Starch Polysaccharides (NSP's, also referred to as crude fiber in the past).

Polysaccharides consist of polymers of simple sugar units or monosaccharides. The monosaccharides are joined by a specific linkage called glycosidic bonds between the hemiacetal group of one sugar and the hydroxyl group of another sugar. The

common glycosidic bonds, α-1-4 and α-1-6 linkages found in starch, α-1-2 bond in sucrose, and β-1-4 link in lactose are cleaved by animal enzyme systems. Most other glycosidic bonds seen in NSP's are unaffected by digestive systems and resist enzymatic action (Smits and Annison, 1996).

Physicochemical properties of the NSP's depend on the solubility of these compounds. NSP's are responsible for increased viscosity of digesta, which can reduce solubility and utilization of nutrients. The importance of solubility and viscosity of NSP's in the digestive tract, which influences nutrient digestion, has been described by Annison (1993). However, he also concluded that attributing antinutritive effects of NSPs solely to the increased viscosity of the intestinal contents may be too simplistic.

Some commonly known NSP's which have importance in poultry diets are raffinose in soybeans, beta-glucans in barley and arabinoxylans in wheat (Leeson and Summers, 2001). Among NSP's, mannans occur in the form of galactomannan, glucomannan, and glucoronomanns in plants (Aman and Graham, 1990). The presence of galactomannas in some protein rich sources like guar meal and copra meal contribute anti-nutritive properties and limit usage of these ingredients in poultry feeds (Carre, 2002).

Guar gum galactomannans

Guar or Cluster bean, (*Cyamopsis tetragonoloba*) is a drought tolerant annual legume indigenous to the Indian subcontinent which is cultivated as a fodder and green manure crop to improve soil fertility. In the early 1950s U.S. commercial production of guar began in north Texas and southwestern Oklahoma. Guar seed consists of hull, germ

and a large endosperm, unlike the seeds of other legumes. The endosperm consists of primarily high molecular weight polysaccharides composed of galactomannans which are linear chains of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains (Figure 2-2). The mannose: galactose ratio is approximately 2:1 (FAO publications 2006).

The seeds are split, dehulled, milled, hydrolyzed and purified to obtain ground endosperm, the native guar gum, which has commercial value. Industrial applications of guar gum include but are not limited to the food industry as a thickening additive, textile printing, explosives, and oil/gas drilling industry (Whistler and Hymowitz, 1979). Guar gum, galactomannan NSP, is not digested in the digestive tract of monogastric animals. Dietary inclusion of pure guar gum at 1% did not produce any significant effect on histomorphology of the small intestine in piglets (Van Nevel et al, 2005). Considerable studies about guar gum have been mainly concentrated on its capacity to improve glucose tolerance levels, and lower blood cholesterol levels in rats (Blackburn and Johnson, 1981; Dario-Frias et al, 1998; Favier et al, 1998).

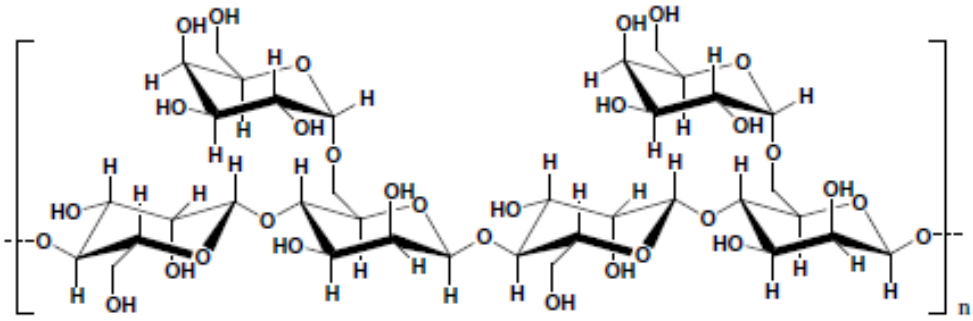

Figure 2-2: Structure of guar gum galactomannan-mannose backbone and galactose side chain (FAO, 2006)

Guar meal, a co-product of guar gum production, is a rich protein source and can be used in animal diets. However, use of guar meal in poultry feeding is limited by its adverse effects on feed intake, growth and production (Curl et al, 1986). Residual guar gum (galactomannan) present in guar meal is probably the major factor responsible for these reported adverse effects (Verma and Mcnab, 1984; Curl et al, 1986; Lee et al, 2003). Other anti-nutrient compounds present in guar meal such as saponins, and possible trypsin inhibitors have been shown to cause decreased production (Curl et al, 1986). Whether residual guar gum or saponin is primarily responsible for the negative effects on the animal performance is not clear. Several investigations reported adverse effects of β-galactomannan found in guar gum and guar meal (Ray et al, 1982; Curl et al, 1986; Lee et al, 2003). Dietary inclusion of guar gum in poultry has been demonstrated to depress growth rate, increased intestinal viscosity associated with delayed gastric emptying, increased length and weight of intestinal tract, increased mortality rate and depressed nutrient utilization (Patel et al, 1980; Ray et al, 1982).

On the other hand, there is some evidence suggesting benefits of feeding guar gum galactomannans to rats. Guar gum has been demonstrated to improve gut health through its prebiotic properties, in addition to its possible cholesterol lowering effect in rats (Dario-Frias et al, 1998; Favier et al, 1998; Moriceau et al, 2000). Adding guar gum to broiler diets has been reported to produce deleterious effects on performance when being fed at a higher concentration of 2% in 2 week old broilers (Daskiran et al, 2004). Daskiran et al, (2004) reported that the negative effects of guar gum supplementation

were partially alleviated by the inclusion of β-mannanase enzyme at 0.05%. Supplementing diets with mannanase enzyme resulted in improved feed utilization.

Alleviating the adverse effects of NSP's such as galactomannan is important to improve energy utilization. Interestingly, the addition of penicillin to poultry rations has been shown to reduce the negative effects associated with guar gum (Patel et al, 1980). As sub-therapeutic antibiotic usage in animal husbandry is no longer desired, supplementation of diets containing galactomannans with exogenous enzymes is a potential strategy to reduce anti-nutritive effects of the NSP's.

Exogenous enzymes

One potential strategy to alleviate the anti-nutritional properties of NSP's is adding exogenous enzymes to the diet. These enzymes cleave NSP's thus resulting in reduced viscosity and enhanced nutrient utilization by the birds. Supplementation of exogenous enzymes such as phytase (to improve phosphorus utilization) is common in poultry feeds. Other enzymes break down indigestible NSP's thereby decreasing the viscosity of digesta and improving digestibility of feed (Bedfored et al, 1991). The use of exogenous enzymes in monogastric animals not only improves digestibility of feed ingredients but also reduces nutrient excreta output thereby offering a possible solution to some of the environmental issues associated with poultry production.

The addition of exogenous enzymes in poultry diets has been well investigated and several commercial enzymes are available in the market. Exogenous enzyme supplementation is known to produce several benefits in poultry production such as improved feed conversion ratio, increase in growth rate, weight gain, and improved

digestibility and reduction in excreta output (Patel and McGinnis, 1985; Annison and Choct, 1991; Campbell and Bedford, 1992; Annison and Choct, 1993; Marquardt et al, 1996; Choct, 2001; Daskiran et al, 2004).

The available literature to date has suggested beneficial effects of dietary prebiotic YCW-MOS, FOS, and the use of exogenous enzymes to improve performance in poultry. Residual guar gum galactomannans present in guar meal have been reported to show improved resistance to *Salmonella* infection in laying hens during molting (Zhang, 2005). To my knowledge, no study has evaluated the prebiotic effects of guar gum galactomannans on intestinal histomorphology and microbial ecology in broilers.

Nutrient intervention strategies are increasingly being considered to enhance gut health. One of the proposed dietary intervention strategies is the use of yeast cell wall containing mannanoligosaccharides (YCW-MOS). For this dissertation, six experiments were conducted to evaluate the effects of different concentrations and different sources of YCW on starting broiler performance using a "challenge model" in which birds are subjected to a Infectious Bursal Disease vaccine and *Clostridium perfringens* challenge. Based on these results, taking YCW-MOS product (Safmannan®) as positive control, a study was designed to evaluate the effects of dietary supplementation of guar gum galactomannans with and without exogenous Mannanase Guar® (β-galactomannanse, 1000 units/gram and cellulose 500 units/gram) enzyme on starting broiler performance in terms of growth, feed conversion, apparent ileal energy digestibility, intestinal histomorphology and microbial ecology.

The hypotheses of this research are: 1) Dietary supplementation of YCW-MOS in starting broilers improves performance under stress conditions; 2) Effectiveness of YCW-MOS depends on both source and concentration; and 3) The prebiotic properties of enzyme (β-galactomannanase) supplemented guar gum galactomannans are equivalent to those of YCW-MOS in starting broilers.

CHAPTER III

EVALUATION OF YEAST CELL WALL MANNANOLIGOSACCHARIDE PRODUCTS IN STARTING BROILERS UNDER IMMUNE STRESS AND *Clostridium perfringens* CHALLENGE

INTRODUCTION

Antimicrobial feed additives have been shown to have a tremendous effect on the growth rate of the animal, feed efficiency and reducing colonization of enteric pathogens (Stutz and Lawton, 1984; Leeson and Summers, 2001). New regulations on the prophylactic use of dietary antibiotic growth promoters in the European Union have accelerated the research to find alternate strategies to improve animal health. Dietary supplementation of probiotics and prebiotics is one possible strategy to enhance host health and to improve productive performance of animals. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confers health benefits on the host" (FAO/WHO, 2002). Several investigations have demonstrated that adding probiotic cultures to poultry diets has beneficial effects on host health by preventing colonization of enteric pathogens (Nurmi and Rantala, 1973; Weinack et al, 1979; Corrier et al, 1995; McReynolds et al, 2009). One limitation with the use of probiotics is that the probiotic organism needs to be established in the host intestine before exerting any beneficial effects on the host. When these probiotic products are withdrawn or no longer consumed, added bacterial populations are quickly washed out of the intestine (Bouhnik et al, 1992).

To overcome the limitations associated with the use of probiotic cultures, the concept of a prebiotic was proposed by Gibson and Roberfroid, (1995). Prebiotics are dietary substances utilized to improve growth rates of the host by targeting beneficial bacteria already colonizing the intestine. A prebiotic is defined as a nondigestible food ingredient that can be utilized by beneficial intestinal microflora thus leading to improved host health (Gibson and Roberfroid, 1995). Nondigestible carbohydrates (both oligosaccharides and polysaccharides) have been proposed as candidate prebiotics. Oligosaccharides are complex carbohydrates consisting of short chain (3-8) monosaccharides. Mannanoligosaccharides (MOS) and fructooligosaccharides (FOS) have both been investigated for prebiotic activities (Ammerman et al, 1989; Kumprecht et al, 1997; Fukata et al, 1999; Spring et al, 2000). With respect to monosaccharides, the ability of mannose to reduce *Salmonella* colonization in broilers is well documented (Oyofo et al, 1989a; Oyofo et al, 1989b; Oyofo et al, 1989c; Spring et al, 2000).

Bio-Mos® (Alltech Inc., Nicholasville, KY) is a prebiotic-type product derived from yeast cell wall. In the previous literature, it is very common to describe yeast cell wall products as MOS, which is not technically correct. Yeast cell wall products contain MOS along with other manno proteins and β-glucans. In a meta-analysis study conducted by Hooge, (2004) MOS supplemented diets were reported to improve growth rate of broilers, with better feed conversion ratio and low mortality rate. Bio-Mos® has been shown to increase intestinal villus height and improved colonization of beneficial bacteria in broilers (Baurhoo et al, 2007). The increased villus height offers a larger surface for nutrient absorption and thereby is associated with increased growth rates.

Morales-Lopez et al, (2010) suggested that the addition of YCW to the diets enhanced gut maturation by increasing the mucosal resistance to microbial translocation.

Supplementation of Bio-Mos® has been demonstrated to decrease colonization of enteric pathogenic bacteria such as *Salmonella* and *E. coli* (Spring et al, 2000), *E.*coli and *Campylobacter* (Baurhoo et al, 2009). Recent research findings also revealed that these YCW-MOS products may be effective in reducing the occurrence of NE lesions in broilers (Hofacre et al, 2003). Geier et al, (2009) reported that Bio-Mos® supplemented diets alter intestinal microbiota of broilers without affecting performance. YCW (AB Vista, Marlborough, Wiltshire, UK) treated diets exhibited better feed conversion ratio and improved humoral immune response against Newcastle disease in broilers (Ghosh et al, 2012). Ghosh et al, (2012) also reported that YCW supplemented diets were able to reduce intestinal *Salmonella* counts in 52-day-old broilers following oral challenge with *Salmonella pullorum.*

Nutrient intervention strategies are increasingly being considered to enhance gut health. One of the proposed dietary intervention strategies is the use of yeast cell wall mannanoligosaccharides (YCW-MOS). Broilers encounter a variety of stress factors in the commercial environment. It is more likely that research experiments are carried out under ideal conditions. The application of results from research studies conducted under experimental conditions may not be appropriate to commercial practice. In this study, broiler performance was evaluated under both experimentally induced pathogen challenged and unchallenged conditions.

A series of six experiments were conducted to evaluate the effect of different concentrations and different sources of YCW-MOS on the performance of starting broilers in birds that were subjected to a compromised immune system induced by vaccination with an infectious bursal disease vaccine (live attenuated virus) followed by *Clostridium perfringens* challenge. The objectives of this study are 1) to investigate the effects of different sources and doses of YCW-MOS products on growth rate and feed conversion ratio in starting broilers; 2) to determine the best MOS-YCW product or combination of products among those; and 3) to determine the optimal concentration or dose of YCW-MOS in starting broilers.

Hypotheses of this study are 1) Dietary supplementation of YCW-MOS in starting broilers improves performance under stress conditions; 2) Effectiveness of YCW-MOS depends on both source and concentration.

MATERIALS AND METHODS

Six experiments or trials were conducted to investigate different yeast products - Safmannan® derived from bakers yeast, Pronady® derived from brewers yeast and BioSaf® which is a live yeast product. All yeast products evaluated in this study were provided by Lesaffre feed additives (Lesaffre International Agricultural product division, Milwaukee, WI, USA). Product data sheets for all products evaluated in this study can be found in the appendix.

Both challenged and unchallenged experiments were conducted at the same time, but in separate buildings during the first 4 trials. During trials 5 and 6, the unchallenged experiments were eliminated. In the challenged experiments, all birds were subjected to

immune stress and *Clostidium perfringens* challenge. All animal handling procedures were approved by Texas A&M University Animal Use Committee.

General procedure for all experiments

Common procedures followed in all the experiments are explained here with specific differences given under each experiment subheading. Ross 308 straight run broiler chicks used in this study were purchased from Sanderson farms, Bryan, TX. Feed and water were offered *ad libitum* with continuous lighting. A basal, industrial type corn soy based broiler starter diet was prepared (Table 3-1). The basal diet was divided into equal sized batches depending on the number of dietary treatments in that particular experiment and each batch supplemented with one of the YCW-MOS products at a specific concentration. Different dietary treatments investigated in each experiment are described below in separate sections. Dietary treatments were randomly assigned to pens such that each treatment was presented at least once for any given vertical row of pens within the Petersime battery brooders. Daily observations were made with regard to general flock condition, temperature, lighting, water, feed, and unanticipated events in the house. Pens were also checked daily for mortality. Pen averages were considered as the unit of measure for the performance phase of all experiments. Birds dying within the first 3 days of experiment were replaced as mortality occurring this early in any experiment was not considered treatment considered treatment related. Bird weights and feed consumption (grams) by pen were recorded at weekly intervals.

Table 3-1: Composition and nutrient content of broiler starter diets used in all experiments in the study

INGREDIENT	PERCENTAGE
Corn	58.434
Soybean meal 48%	34.493
Dl-Met 98%	0.231
Lysine HCl	0.177
AV Fat, blended	2.755
Limestone	1.561
Mono-Dicalcium Phosphate	1.537
Salt	0.512
Trace minerals premix[1]	0.050
Vitamin premix[2]	0.250
CALCULATED NUTRIENT CONTENT (%)	
Protein	22.00
ME (Kcal/Kg)	3050.00
Crude fat	5.32
Crude fiber	2.63
Calcium	0.95
AV Phosphate	0.71
Sodium	0.22
Methionine	0.56
Lysine	1.31

[1] Trace minerals premix added at this rate yields: 27.50 mg sulphur, 150 mg manganese, 16.50 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, and 0.84 mg molybdenum per kilogram diet.

[2] Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,858 IU vitamin D3, 1.47 mg menadione, 2.90 mg thiamin, 5.80 mg riboflavin, 20 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 478 mg choline, 16.50 μg Vitamin B12, 46.00 mg niacin, and 7.20 mg pyridoxine per kilogram of diet.

Table 3-2: Study design for both the challenged and unchallenged experiments

	Challenged*					Unchallenged**		
Experiment	**Treatments[1]**	**Bird[2]**	**Birds per pen**	**Vaccine[3]**	***C. perfringens* challenge**	**Treatments[1]**	**Bird[2]**	**Birds per pen**
Experiment 1	9	240	5	day 15	Day 18, 19, and 20	5	144	6
Experiment 2	6	240	5	day 15	Day 18, 19, and 20	6	120	6
Experiment 3	9	240	5	day 15	Day 18, 19, and 20	5	120	6
Experiment 4	8	240	5	day 15	Day 18, 19, and 20	4	120	6
Experiment 5	8	288	6	day 10	Day 16, 17, and 18	------Eliminated------		
Experiment 6	8	288	6	day 10	Day 16, 17, and 18			

[1]Total number of dietary treatments in that particular experiment.
[2]Total number of birds in that particular experiment.
[3] Birds in experiment 1 received Cocci-Vac and birds in rest of the experiments received infectious bursal disease vaccine.
*Challenged group have 48 pens in all experiments and all birds received vaccine and challenge.
**Unchallenged group have 24 pens in all experiments.

Performance variables evaluated in this study were final body weight per bird (BW), weight gain per bird (WG), total feed consumption (FC), feed conversion ratio (FCR), Productivity Index (PI), and percent mortality rate (MORT). Productivity index was calculated using the following mathematical formula:

PI = (100-MORT) x (BW/1000)/Bird age/FCR x 100

The study design followed for both challenged and unchallenged is given in the Table 3-2.

Challenged experiments

Birds were distributed among 48 pens in 2 Petersime battery brooder units. Number of dietary treatments, total number of birds, and age at which birds were challenged are illustrated in Table 3-2. The challenge model used to induce Necrotic Enteritis in starting broilers was adapted from McReynolds et al, (2004).

Vaccine administration: A commercial infectious bursal disease (IBD) vaccine (Schering-Plough Animal Health, Millsboro, DE) was used as an immunosuppressant in all experiments except for the first experiment, in which Cocci-Vac® (Schering-Plough Animal Health, Millsboro, DE) was given. Birds were vaccinated with Cocci-Vac® by spraying the prescribed amount directly onto the feed provided to each pen of chickens. The IBD Vaccine was given at a level 10x the recommended dose of the manufacturer to immunocompromise the birds. Each bird in challenged group received the IBD vaccine via ocular route (eye drops).

***Clostridium perfringens* challenge:** Field isolates of *C. perfringens* (Georgia and Texas combined cultures) known to cause Necrotic Enteritis, originating from commercial

flocks were isolated, cultured separately, and then combined (McReynolds et al, 2004). The isolates were grown in thioglycollate medium for 12 h, and fresh inoculum was administered each day. The titration levels were approximately 1.0 x 10^{8-9}. Each bird in challenged groups received *C. perfringens* challenge (2 mL administered by oral gavage to the crop) on days as illustrated in Table 3-2.

Necrotic Enteritis lesion scoring: After 3 wk, all birds which did not previously die were euthanized and visually examined for signs of Necrotic Enteritis using a 0-4 scoring system with zero being normal and 4 being the most severe form as described by Prescott et al, (1978): 0 = normal healthy intestine, no evidence of gross lesions; 1 = thin, friable small intestine, gray appearance; 2 = focal necrosis, ulceration, thin walled, gray appearance; 3 = sizable patchy necrosis, noticeable gas production in small intestine, small hemorrhage; 4 = severe extensive necrosis, large hemorrhages (as seen in birds died from NE), large amounts of gas in small intestine.

Unchallenged experiments

Birds were randomly distributed among 24 pens in one Petersime battery brooder unit. Number of dietary treatments and total number of birds, are illustrated in the Table 3-2. Based on the data from 4 experiments, the unchallenged experiments were eliminated for experiments 5 and 6.

Experiment 1

Two dietary additives studied in this experiment were YCW-MOS Safmannan® and Partially-hydrolyzed Safmannan (PH safmannan) at the rate of 0 (control), 125, 250, 375, and 500 ppm for a total of 9 dietary treatments. A total of 240 broiler chickens were

randomly distributed 48 pens with 5 birds per pen in the challenged group (48 pens) and 144 birds over 24 pens in with 6 birds per pen in the unchallenged group (24 pens). Dietary treatments are illustrated in Table 3-3.

Statistics: For the challenge group, data were analyzed by ANOVA using the General Linear Model (GLM) as a 2 x 5 factorial based on 2 additives, 5 doses of each additive and also analyzed for the interaction of additive and dose. The unchallenged group (Safmannan only) data were analyzed by ANOVA with 5 treatments. Treatment means were separated using Duncan's Multiple Range Test at a P value <0.05.

Experiment 2

The YCW-MOS product, Pronady® (at 125 and 250 ppm), a live yeast extract concentrate, Biosaf® (at 1000 ppm), and a combination of Biosaf and Pronady (at 1000+125 and 1000+250 ppm respectively) were evaluated in this experiment. There were 8 challenged replicates and 4 unchallenged replicates per treatment (Table 3-4).

Statistics: The challenged group data were analyzed by ANOVA with 6 treatments. The unchallenged group data were analyzed by ANOVA with 6 treatments. To examine the effect of challenge, data were also analyzed as challenged group versus unchallenged group using ANOVA without including treatment in the model. Treatment means were separated using Duncan's Multiple Range Test at a P value <0.05.

Experiment 3

Effects of dietary supplementation of two additives Pronady® and Safmannan® at a concentration of 0, 125, 250, 375, and 500 ppm were investigated in this experiment. Dietary treatments and replicates are described in Table 3-5.

Table 3-3: Experimental design for experiment 1

No	Additive	Concentration (ppm)	Challenged Replicates	Unchallenged Replicates
1	PH Safmannan[1]	125	5	0
2	PH Safmannan[1]	250	5	0
3	PH Safmannan[1]	375	5	0
4	PH Safmannan[1]	500	5	0
5	Control	0	8	4
6	Safmannan[2]	125	5	5
7	Safmannan[2]	250	5	5
8	Safmannan[2]	375	5	5
9	Safmannan[2]	500	5	5

[1]PH Safmannan is Safmannan that had been treated by the manufacturer to partially hydrolyze the YCW.
[2]Safmannan is a YCW-MOS product derived from the bakers yeast.

Table 3-4: Experimental design for experiment 2

No	Additive	Concentration(ppm)	Challenged Replicates	Unchallenged Replicates
1	No additive	0	8	4
2	BioSaf[1]	1000	8	4
3	Pronady[2]	125	8	4
4	Pronady[1]	250	8	4
5	BioSaf+Pronady[3]	1000+125	8	4
6	BioSaf+Pronady[3]	1000+250	8	4

[1]BioSaf is a live yeast (*Saccharomyces cerevisiae*) product used in the baking industry.
[2]Pronady is a YCW-MOS product derived from the yeast (*Saccharomyces cerevisiae*) used in the brewing industry.
[3]A combination treatment consisting of Pronady and Biosaf.

Table 3-5: Experimental design for experiment 3

No	Additive	Concentration (ppm)	Challenged Replicates	Unchallenged Replicates
1	Control	0	8	4
2	Pronady[1]	125	5	5
3	Pronady[1]	250	5	0
4	Pronady[1]	375	5	5
5	Pronady[1]	500	5	0
6	Safmannan[2]	125	5	5
7	Safmannan[2]	250	5	0
8	Safmannan[2]	375	5	5
9	Safmannan[2]	500	5	0

[1]Pronady is a YCW-MOS product derived from the brewers yeast.
[2]Safmannan is a YCW-MOS product derived from the bakers yeast.

Table 3-6: Experimental design for experiment 4

No	Additive	Concentration (ppm)	Challenged Replicates	Unchallenged Replicates
1	No additive	0	6	6
2	Pronady[1]	125	6	0
3	Pronady[1]	250	6	6
4	Safmannan[2]	125	6	0
5	Safmannan[2]	250	6	6
6	Safmannan[2]	500	6	0
7	P80S20[3]	125	6	0
8	P80S20[3]	250	6	6

[1]Pronady is a YCW-MOS product derived from the brewers yeast.
[2]Safmannan is a YCW-MOS product derived from the bakers yeast.
[3]P80S20 is a blend of 80% of Pronady and 20% Safmannan.

Statistics: Data were analyzed by ANOVA using the GLM procedure as a 2 x 5 factorial based on two sources and 5 doses of each source and also for the interaction between source and dose. Treatment means were separated using Duncan's Multiple Range Test at a P value <0.05.

Experiment 4

Feed was supplemented with one of the two additives (Pronady® at 125 and 250 ppm or Safmannan® at 125, 250 and 500 ppm). Two additional treatments consisted of a blend of 80% Pronady with 20% Safmannan at a total final concentration of 125 or 250 ppm. Dietary treatments and replicates are illustrated in Table 3-6.

Statistics: Data were analyzed by ANOVA using the GLM procedure, based on 8 treatments and as an imbedded 2 x 2 factorial based on the two sources and 2 doses of each source, and also for the interaction of source and dose. Treatment means for Dose were separated using Duncan's Multiple Range Test at a P value <0.05.

Experiment 5

The basal diet was supplemented with one of two additives (Pronady® or **S**afmannan®) at the rate of 0, 125, 250, or 500 ppm. One additional treatment consisted of a blend of 50% Pronady and 50% Safmannan at a total final concentration of 134 ppm. This experiment was terminated on day 20, one day earlier than previous experiments because of high mortality. Treatments and replicates are given in Table 3-7.

Table 3-7: Experimental design for experiment 5

No	Treatment	Concentration (ppm)	Challenged Replicates
1	No additive	0	6
2	Pronady[1]	125	6
3	Pronady[1]	250	6
4	Pronady[1]	500	6
5	Safmannan[2]	125	6
6	Safmannan[2]	250	6
7	Safmannan[2]	500	6
8	P50S50[3]	134	6

[1]Pronady is a YCW-MOS product derived from the brewers yeast.
[2]Safmannan is a YCW-MOS product derived from the bakers yeast.
[3]P50S50 is a blend of 50% of Pronady and 50% Safmannan.

Table 3-8: Experimental design for experiment 6

No	Additive	Concentration	Challenged Replicates
1	No additive	0	6
2	FR Safmannan[1]	250	6
3	CR Pronady[2]	250	6
4	BR Pronady[3]	250	6
5	FR S50+CR P50[4]	250	6
6	FR S50+BR P50[5]	250	6
7	CR P50+BR P50[6]	250	6
8	FR S33.3+BR P33.3+CR P33.3[7]	250	6

[1]FR Safmannan is a YCW-MOS product derived from the bakers yeast and manufactured in France.
[2]CR Pronady is a YCW-MOS product derived from the brewers yeast and manufactured in Cedar Rapids, Iowa, USA.
[3]BR Pronady is a YCW-MOS product derived from the brewers yeast and manufactured in Brazil.
[4]FR S50+CR P50 is a blend of 50% FR Safmannan and 50% CR Pronady.
[5]FR S50+BR P50 is a blend of 50% FR Safmannan and 50% BR Pronady.
[6]CR P50+BR P50 is a blend of 50% CR Pronady and 50% BR Pronady.
[7] FR S33.3+BR P33.3+CR P33.3 is a blend of 33.3% FR Safmannan, 33.3% BR Pronady and 33.3% CR Pronady.

Statistics: Data were analyzed by ANOVA using the GLM procedure and as an imbedded 2 x 3 factorial based on the two sources and 3 doses of each source, and also for interaction of source and dose. Treatment means for Dose were separated using Duncan's Multiple Range Test P value <0.05.

Experiment 6

This experiment investigated YCW-MOS products from various sources (different manufacturing plants) individually and in combination. All products were evaluated at 250 ppm. The basal diet was supplemented with one of the three additives or a blend (BR Pronady® from Brazil, CR Pronady® from Cedar Rapids, Iowa, USA; and FR Safmannan® from France) as indicated under the experimental design in Table 3-8.

Statistics: Data were analyzed by ANOVA using the GLM procedure based on 8 treatments. Treatment means were separated using Duncan's Multiple Range Test P value <0.05.

Pooled data analysis: 6 challenged experiments

Data obtained from 6 challenged experiments were pooled and analyzed for the main effects of source (Control, Pronady®, Safmannan® and blend), dose (0, 125, 250, 375 and 500) and for the interaction of source and dose using the GLM procedure by including experiment as a fixed factor. The Biosaf® 1000 ppm treatment from experiment 2 was excluded from the data and partially hydrolyzed safmannan treatment from experiment 1 was considered as a safmannan treatment. In all challenged experiments, data from broilers receiving any concentration of Safmannan, Pronady and

blend were evaluated for the effect of source and data from birds receiving 0, 125, 250, 375 and 500 ppm of YCW-MOS additive were analyzed for the effect of dose. Variables analyzed were body weight per bird, weight gain per bird, total feed consumption, FCR, PI, and percent mortality.

Pooled data analysis: 4 unchallenged experiments

Data collected from the 4 unchallenged experiments were pooled and analyzed for the main effects of source (control, Pronady®, Safmannan® and blend) and dose (0, 125, 250, 375 and 500 ppm) using the GLM procedure by including experiment as a fixed factor. Biosaf® 1000 ppm treatment from experiment 2 was excluded from the data. No significant differences were found in pooled data analysis of unchallenged experiments so the pooled unchallenged experiments are not discussed further.

RESULTS

Experiment 1

In the challenged group, there was no effect of dietary additive (Safmannan and PH Safmannan) on any of the variables tested (data not shown). There was no significant interaction between source and dose. However, dose had a significant beneficial effect at a concentration of 250 ppm or more on feed conversion ratio (FCR, feed intake to weight gain ratio), productivity index (PI) and percent morality (Table 3-9). When YCW-MOS was included at 375 ppm total percent mortality was significantly reduced compared to the control. No differences were observed in NE lesion score. In the unchallenged group, none of the treatments had significant effects on body weight, weight gain, feed consumption, FCR, PI, or mortality (Table 3-10).

Table 3-9: Effects of different doses of YCW-MOS supplementation on performance in the challenged group of broilers (21-day-old) from experiment 1

Dose(ppm)	n	BW*	WG*	FC*	FCR	PI	NEL	MORT(%)
0	8	812	772	1313	1.70^{b}	230^{b}	0.27	12.5^{b}
125	9	826	785	1270	1.62^{ab}	244^{ab}	0.24	11.1^{ab}
250	9	858	816	1241	1.52^{a}	270^{a}	0.35	2.2^{ab}
375	9	841	800	1185	1.48^{a}	271^{a}	0.29	0.0^{a}
500	9	825	784	1210	1.55^{a}	255^{ab}	0.38	6.7^{ab}
PSEM**		17.64	17.62	44.13	0.05	10.84	0.11	3.72
P value		0.48	0.50	0.30	0.04	0.06	0.88	0.09

^{a,b}Means with no common superscript in the same row differ significantly (P<0.05).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Table 3-10: Effects of different doses of YCW-MOS supplementation on performance in the unchallenged group of broilers (21-day-old) from experiment 1

Dose(ppm)	n	BW*	WG*	FC*	FCR	PI	MORT(%)
0	4	779	734	1133	1.54	262	8.33
125	4	770	729	1090	1.50	232	0.00
250	4	737	696	1052	1.52	249	4.17
375	4	787	746	1089	1.46	273	0.00
500	5	796	755	1112	1.47	193	0.00
PSEM**		30.60	30.66	39.48	0.04	30.52	5.39
P value		0.70	0.70	0.69	0.65	0.36	0.50

^{a,b}Means with no common superscript in the same row differ significantly (P<0.05).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Table 3-11: Effects of different doses of YCW-MOS supplementation on performance in the challenged group of broilers (21-day-old) from experiment 2

Treatment	n	BW*	WG*	FC*	FCR	PI	NEL	MORT(%)
0 Control	8	642^{c}	600^{c}	1105^{b}	1.55^{a}	182^{c}	0.88^{b}	17.5^{ab}
1000 ppm BioSaf[1]	8	745^{b}	701^{b}	1117^{b}	1.49^{ab}	223^{b}	1.57^{a}	27.5^{a}
125 ppm Pronady[2]	8	844^{a}	800^{a}	1209^{a}	1.42^{ab}	267^{a}	0.96^{b}	10.0^{b}
250 ppm Pronady	8	850^{a}	807^{a}	1190^{a}	1.40^{b}	275^{a}	1.01^{b}	7.5^{b}
1000 ppm BS+125 P[3]	8	837^{a}	794^{a}	1151^{a}	1.37^{b}	275^{a}	0.86^{b}	2.5^{b}
1000 ppm BS+250 P[4]	8	823^{a}	780^{a}	1195^{a}	1.45^{ab}	257^{a}	1.22^{ab}	10.0^{b}
PSEM**		20.96	20.98	45.44	0.05	11.49	0.17	5.29
P value		<0.001	<0.001	<0.001	0.041	<0.001	0.062	0.030

[1]BioSaf is a live yeast (*Saccharomyces cerevisiae*) product used in the baking industry.
[2]Pronady is a YCW-MOS product derived from the yeast (*Saccharomyces cerevisiae*) used in the brewing industry.
[3]Diets supplemented with 1000 ppm BioSaf and 125 ppm Pronady.
[4]Diets supplemented with 1000 ppm BioSaf and 250 ppm Pronady.
[a,b]Means with no common superscript in the same row differ significantly ($P<0.05$).
* Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Table 3-12: Effects of different doses of YCW-MOS supplementation on performance in the unchallenged group of broilers (21-day-old) from experiment 2

Treatment	n	BW*	WG*	FC*	FCR	PI	MORT(%)
0 Control	4	701^{a}	658^{a}	958^{b}	1.46^{ab}	230^{a}	0.0^{a}
1000 ppm BioSaf[1]	4	836^{b}	793^{b}	1216^{a}	1.53^{a}	258^{b}	20.0^{b}
125 ppm Pronady[2]	4	862^{b}	818^{b}	1136^{a}	1.39^{b}	296^{cd}	0.0^{a}
250 ppm Pronady	4	887^{b}	843^{b}	1159^{a}	1.37^{b}	307^{d}	0.0^{a}
1000 ppm BS+125 P[3]	4	848^{b}	805^{b}	1116^{a}	1.38^{b}	291^{cd}	0.0^{a}
1000 ppm BS+250 P[4]	4	846^{b}	802^{b}	1197^{a}	1.49^{ab}	270^{bc}	10.0^{ab}
PSEM**		17.55	17.53	43.97	0.04	8.92	5.27
P value		<0.001	<0.001	<0.001	0.069	<0.001	0.060

[1]BioSaf is a live yeast (*Saccharomyces cerevisiae*) product used in the baking industry.
[2]Pronady is a YCW-MOS product derived from the yeast (*Saccharomyces cerevisiae*) used in the brewing industry.
[3]Diets supplemented with 1000 ppm BioSaf and 125 ppm Pronady.
[4]Diets supplemented with 1000 ppm BioSaf and 250 ppm Pronady.
[a,b]Means with no common superscript in the same row differ significantly (P<0.05).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Experiment 2

In this challenged experiment, Biosaf® (live yeast) and Pronady® (Yeast Cell Wall Product) effects were investigated at different dietary concentrations. All dietary treatments showed significant improvement in body weight, weight gain, and PI compared to the control (Table 3-11). Birds being fed with Pronady at 250 ppm displayed greater body weight, weight gain, higher feed consumption, better FCR, and higher PI compared to the control, Biosaf 1000 ppm or Pronady 125 ppm treatments. Live yeast (Biosaf) doesn't seem to be effective against *C. perfringens* challenge when compared to Pronady even though it was significantly better from the control with respect to body weight, weight gain and productivity index.

Results for the unchallenged group are shown in Table 3-12. All dietary treatments resulted in better performance compared to the control. Without challenge, Biosaf resulted in greater body weight but not without an increase in FCR, which is not desirable. Overall, dietary supplementation of Pronady at 250 ppm produced better results even in the unchallenged group. Significant improvements were observed in unchallenged group for the production variables FCR, PI and percent mortality compared to the challenged group (Table 3-13).

Table 3-13: Effects of challenge on 21-day-old broiler performance receiving YCW-MOS supplemented diets in experiment 2

	n	BW*	WG*	FC*	FCR	PI	MORT(%)
Challenged	48	790	747	1145	1.45[a]	247[b]	12.5[a]
Unchallenged	24	830	787	1130	1.36[b]	275[a]	5.0[b]
PSEM**		15.31	15.28	23.49	0.02	7.36	2.66
P value		0.071	0.071	0.669	0.006	0.007	0.05

[a,b]Means with no common superscript in the same column differ significantly (P<0.05).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Table 3-14: Effects of source and dose of YCW-MOS supplementation on performance in the challenged group of broilers (21-day-old) from experiment 3

	n	BW*	WG*	FC*	FCR	PI	NEL	MORT
Source								
Safmannan[1]	24	811	772	1411	1.81	216	1.15	26.95
Pronady[2]	23	799	760	1317	1.74	225	1.16	20.00
PSEM**		12.81	12.76	62.88	0.08	9.56	0.10	4.02
Dose								
0	8	697[b]	658[b]	1185	1.80	189	0.92	15.00
125 ppm	10	839[a]	800[a]	1417	1.78	230	1.41	26.00
250 ppm	10	805[a]	766[a]	1396	1.82	217	1.24	28.00
375 ppm	10	843[a]	804[a]	1536	1.89	215	1.13	32.00
500 ppm	9	821[a]	783[a]	1233	1.57	249	0.99	13.33
PSEM**		20.33	20.24	99.82	0.12	15.17	0.17	6.39
P value								
Source		0.485	0.486	0.362	0.622	0.614	0.919	0.270
Dose		0.0001	0.0001	0.1180	0.5020	0.1510	0.3130	0.1770
Sourse*Dose		0.760	0.742	0.553	0.579	0.686	0.759	0.728

[1]YCW-MOS product from bakers yeast.
[2]YCW-MOS product from brewers yeast.
[a,b,c]Means for main effects in a column with no common superscript differ (P<0.05).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Experiment 3

In the challenged group, no significant effects of source (Pronady® and Safmannan®) were detected (Table 3-14). There was no significant interaction between source and dose. However, dose significantly improved body weight and weight gain at all the concentrations tested (Table 3-14). No significant differences were detected for feed intake, FCR, PI, NEL and percent mortality (Table 3-14). No significant differences were observed for any of the production variables in the unchallenged group of birds (data not shown).

Experiment 4

There were no significant differences detected for any of the parameters measured in this experiment, either in the challenged (Table 3-15) or the unchallenged (Table 3-16) groups. No significant differences were observed in 2 x 2 factorial analyses (Pronady and Safmannan at 125 and 250 ppm) (data not shown). No significant interaction between source and dose was detected.

Table 3-15: Effects of different YCW-MOS product supplementation on performance in the challenged group of broilers (21-day-old) from experiment 4

Treatment	n	BW*	WG*	FC*	FCR	PI	MORT(%)	NEL
0	6	880	836	1387	1.68	263	6.67	1.22
Pronady125[1]	6	937	893	1232	1.38	324	3.33	0.90
Proandy250[1]	6	914	871	1267	1.46	302	0.00	0.86
Safmannan125[2]	6	906	862	1275	1.48	296	0.00	1.19
Safmannan250[2]	6	968	924	1350	1.47	320	3.33	1.07
Safmannan500[2]	6	928	884	1351	1.53	293	0.00	1.01
P80S20-125[3]	6	902	858	1234	1.44	301	0.00	1.32
P80S20-250[3]	6	940	895	1301	1.45	310	0.00	1.06
PSEM**		26.15	26.22	72.4	0.08	17.54	2.23	0.24
P value		0.390	0.400	0.722	0.420	0.236	0.290	0.879

[1] Diets containing Pronady (YCW-MOS product derived from the brewers yeast) at a given concentration.
[2] Diets containing Safmannan (YCW-MOS product derived from the bakers yeast) at a given concentration.
[3] Diets containing a blend of Pronady 80 and Safmannan 20 (blend of 80% of Pronady and 20% Safmannan) at a given final concentration.
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Table 3-16: Effects of different YCW-MOS product supplementation on performance in the unchallenged group of broilers (21-day-old) from experiment 4

Treatment	n	BW*	WG*	FC*	FCR	PI	MORT(%)
Control	6	911	869	1273	1.47	297	0.00
Pronady250[1]	6	910	867	1276	1.48	295	0.00
Safmannan250[2]	6	872	828	1232	1.49	280	0.00
P80S20-250[3]	6	885	842	1216	1.45	291	0.00
PSEM**		30.96	30.96	34.54	0.02	13.11	0
P value		0.408	0.408	0.536	0.645	0.354	0

[1] Diets containing Pronady (YCW-MOS product derived from the brewers yeast) at a given concentration.
[2] Diets containing Safmannan (YCW-MOS product derived from the bakers yeast) at a given concentration.
[3] Diets containing a blend of Pronady 80 and Safmannan 20 (blend of 80% of Pronady and 20% Safmannan) at a given final concentration.
*Final body weight, weight gain and total total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Experiment 5

Broilers receiving the combination treatment (50:50 blends of Pronady® and Safmannan® at a final concentration of 134 ppm) weighed significantly more than those of other dietary treatments evaluated in this experiment (Table 3-17). FCR and PI were also significantly better for this combination treatment. However, no difference in feed consumption was detected. This particular population of birds was significantly smaller compared to those of other experiments. They most likely came from lightweight broiler breeders just entering production. In this experiment, challenge was accelerated to see if it might reduce the variability (randomness) associated with the NE lesion scoring. The accelerated challenge did not lower the variation of the NE lesion scoring. Mortality was quite a bit higher than normal in this experiment, so it was decided to terminate the experiment 1 day early (day 20 Versus 21). There was some visual evidence of lesion healing, but this could not be related to any particular treatment. No significant differences were detected by the 2 x 3 factorial analysis (Pronady and Safmannan at 125, 250 and 500 ppm) except for productivity index (data not shown). Safmannan has significantly higher productivity index compared to Pronady.

Table 3-17: Effects of YCW-MOS supplementation on performance in the challenged group of broilers (20-day-old) from experiment 5

Treatment	n	BW*	WG*	FC*	FCR	PI	MORT(%)	NEL
Control	6	722^{bc}	678^{bc}	1029	1.52^{a}	220^{c}	13.89	1.16
Pronady125[1]	6	751^{abc}	707^{abc}	1023	1.45^{ab}	247^{abc}	19.45	1.36
Pronady250[1]	6	718^{c}	674^{c}	1017	1.52^{a}	221^{c}	19.44	1.48
Pronady500[1]	6	747^{abc}	704^{abc}	1056	1.50^{ab}	231^{bc}	8.33	1.72
Safmannan125[2]	6	786^{abc}	742^{abc}	1079	1.45^{ab}	250^{abc}	22.22	1.29
Safmannan250[2]	6	794^{ab}	750^{ab}	1088	1.45^{ab}	261^{ab}	16.67	1.23
Safmannan500[2]	6	745^{abc}	701^{abc}	1030	1.47^{ab}	242^{abc}	13.89	1.67
P50S50[3]	6	819^{a}	775^{a}	1103	1.42^{c}	269^{a}	25.00	1.23
PSEM**		23.02	23.00	30.77	0.02	11.42	7.27	0.24
P value		0.035	0.036	0.337	0.102	0.103	0.805	0.621

[1] Diets containing Pronady (a YCW-MOS product derived from the brewers yeast) at a given concentration.
[2] Diets containing Safmannan (a YCW-MOS product derived from the bakers yeast) at a given concentration.
[3]P50S50 is a blend of 50% of Pronady and 50% Safmannan with a final concentration of 134 ppm.
[a,b,c] Means within a column with no common superscript differ significantly ($P<0.05$).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.

Table 3-18: Effects of YCW-MOS supplementation on performance in the challenged group of broilers (21-day-old) from experiment 6

Treatment**	n	BW*	WG*	FC*	FCR	PI	MORT(%)
Control	6	819[a]	773[a]	1207	1.57[b]	194	22.2
FR Safmannan[1]	6	841[ab]	796[ab]	1202	1.52[b]	197	27.7
CR Pronady[2]	6	844[ab]	798[ab]	1202	1.51[b]	233	13.9
BR Pronady [3]	6	856[ab]	811[ab]	1238	1.53[b]	224	16.6
FR S50+CR P50[4]	6	923[b]	877[b]	1206	1.38[a]	267	16.7
FR S50+BR P50[5]	6	891[ab]	846[ab]	1237	1.46[ab]	226	22.2
CR P50+BR P50[6]	6	897[ab]	851[ab]	1250	1.47[ab]	235	19.4
FR S33.3+BR P33.3+CR P33.3[7]	6	892[ab]	846[ab]	1308	1.55[b]	202	27.8
PSEM* **		28.21	28.24	44.93	0.04	24.7	7.53
P value		0.170	0.178	0.708	0.081	0.459	0.853

[1]FR Safmannan is a YCW-MOS product derived from the bakers yeast and manufactured in France.
[2]CR Pronady is a YCW-MOS product derived from the brewers yeast and manufactured in Cedar Rapids, Iowa, USA.
[3]BR Pronady is a YCW-MOS product derived from the brewers yeast and manufactured in Brazil.
[4]FR S50+CR P50 is a blend of 50% FR Safmannan and 50% CR Pronady.
[5]FR S50+BR P50 is a blend of 50% FR Safmannan and 50% BR Pronady.
[6]CR P50+BR P50 is a blend of 50% CR Pronady and 50% BR Pronady.
[7] FR S33.3+BR P33.3+CR P33.3 is a blend of 33.3% FR Safmannan, 33.3% BR Pronady and 33.3% CR Pronady.
[a,b] Means within a column with no common superscript differ significantly ($P<0.05$).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**All the dietary treatments were supplemented at a concentration of 250 ppm.
***Pooled standard error mean.

Experiment 6

YCW-MOS products manufactured in different plants and their combinations were tested in this experiment. Dietary treatments with the FR S50+CR P50 combination performed significantly better than the control with greater body weight, weight gain and low FCR (Table 3-18). Performance variables tested are shown in Table 3-18.

Pooled data analysis

Pooled data analysis from the challenged experiments revealed that the source and dose of YCW-MOS additive had significant beneficial effects on the performance (Table 3-19). No significant differences were detected between the two sources (Pronady® and Safmannan®) of YCW-MOS. There was no significant interaction between source and dose. Safmannan and Pronady performed significantly better compared to the control and resulted in an overall improvement of 10% growth rate with no difference in FCR. The blend (mix of Pronady and Safmannan) produced better effects with an improvement of more than 15% growth rate, more than 10% reduction in FCR, greater feed consumption and higher productivity index compared to the control. All doses of YCW-MOS displayed significant improvement compared to the control and produced a greater body weight with significantly lower FCR. A dose effect of YCW-MOS on body weight was demonstrated in Figure 3-1, with a peak response close to 250 ppm and a decline in body weight above 300 ppm. No significant differences in mortality rate were observed.

Table 3-19: Effects of source and dose of YCW-MOS product on performance in 21-day-old broilers (Pooled data from all 6 challenged experiments excluding the Biosaf treatment from experiment 2)

	n	BW*	WG*	FC*	FCR	PI	MORT(%)
Source							
Control	42	756^{c}	713^{c}	1160^{b}	1.63^{a}	199^{c}	16.03
Safmannan[1]	98	841^{b}	799^{b}	1206^{ab}	1.51^{ab}	242^{b}	14.49
Pronady[2]	78	829^{b}	786^{b}	1162^{b}	1.48^{bc}	245^{ab}	14.36
Blend[3]	58	877^{a}	832^{a}	1217^{a}	1.46^{c}	261^{a}	14.2
PSEM**		8.22	8.21	18.62	0.02	5.94	1.96
Dose							
0	36	745^{c}	703^{c}	1152	1.64^{a}	200^{c}	15.00
125	37	844^{a}	801^{a}	1182	1.48^{b}	259^{a}	13.43
250	71	858^{a}	814^{a}	1212	1.49^{b}	244^{ab}	15.69
375	19	842^{a}	802^{a}	1206	1.50^{b}	222^{b}	16.84
500	113	809^{b}	767^{b}	1159	1.51^{b}	240^{ab}	12.16
PSEM**		10.48	10.48	23.77	0.02	7.57	2.50
P value							
Source		0.053	0.055	0.333	0.209	0.144	0.775
Dose		0.427	0.435	0.231	0.706	0.762	0.463
Sourse*Dose		0.432	0.436	0.538	0.600	0.299	0.515

[1]YCW-MOS product from bakers yeast.
[2]YCW-MOS product from brewers yeast.
[3]Mix of Safmannan and Pronady.
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Pooled standard error mean.
[a,b,c]Means for main effects in a column with no common superscript differ (P<0.05).

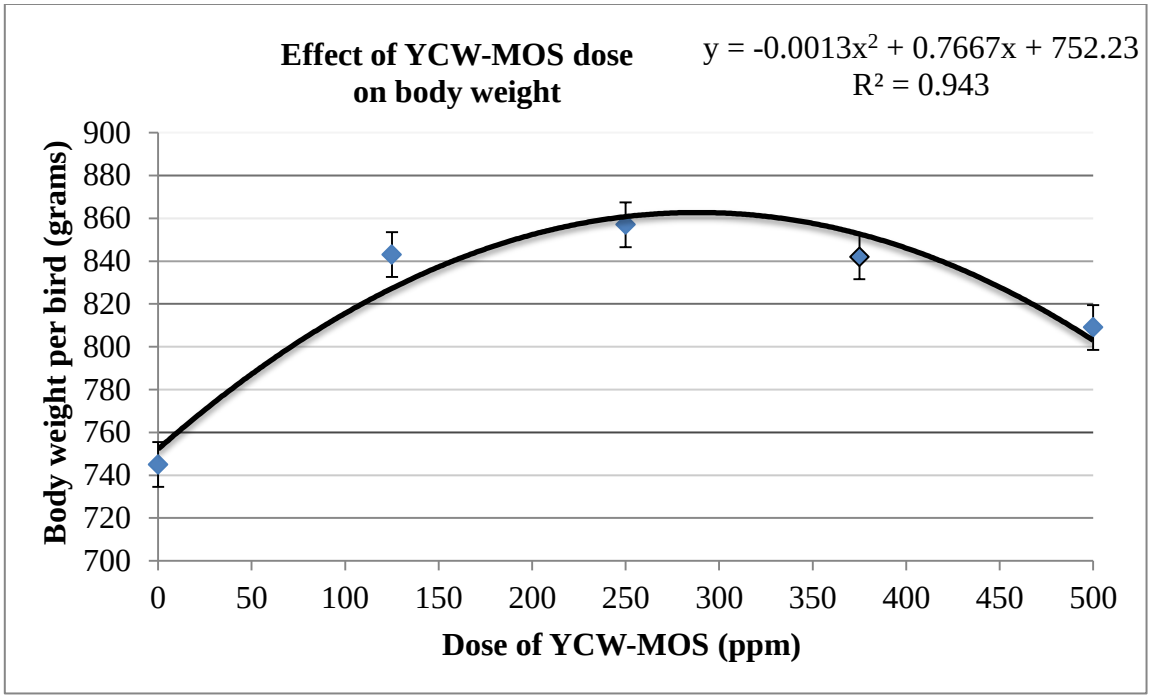

Figure 3-1: Effect of YCW-MOS dose on body weight in 21-day-old broilers

DISCUSSION

This study indicated that dietary inclusion of YCW-MOS improved body weight, weight gain, and productivity index and reduced FCR in starting broilers under immune stress and *Clostridium perfringens* challenge. The improved weight gain in supplemented broilers is likely due to the prebiotic functionality of YCW-MOS, which has been reported to promote colonization of beneficial bacteria, improve intestinal integrity, and enhance immune functions (Ferket et al, 2002; Loddi et al, 2002; Yang et al, 2008; Ghosh et al, 2012). In this study, no difference in production parameters were observed between the two sources of YCW-MOS product tested (Safmannn® – The baker's yeast product and Pronady® – The brewer's yeast product), however the blended product (mix of safmannan and pronady) produced a greater body weight with no difference in FCR compared to the individual YCW products. There is a distinct possibility that the blended products are performing better than the unblended products. This may be due to a "broader" immune response from the mannan proteins present in the YCW-MOS products (the outer surface of YCW contains mannoproteins, which are covalently linked to β-glucans on the inner layer). Even though YCW-MOS products tested in this study were derived from the same yeast species, *Saccharomyces cerevisiae*, bakers yeast and brewers yeast likely have different mannan proteins, stimulating a broader immune response.

It has been reported that YCW-MOS products did not produce consistently beneficial results on broiler production performance. The current results were in accordance with some previous reports and contradictory to other reports. Gao et al,

(2008) described that oligosaccharides supplemented in diets increased feed intake in broilers by improving appetite. Increased feed consumption eventually results in greater body weight gain and can reduce FCR, which support the findings of the current study. The current study also revealed that the optimum level of YCW-MOS products (LeSaffre feed additives, Milwaukee, WI) in starting broiler diets is approximately 250 ppm. It was reported that diets supplemented with Safmannan® at a rate of 500 ppm did not affect the body weight, FCR or mortality in starting, growing or finishing broilers under normal conditions (Benites et al, 2008). In the unchallenged group of present study, YCW-MOS produced no significant improvement in growth rate.

In contrast to the results presented here, Bio-Mos® at 1000 ppm in starter feed and 500 ppm in grower and finisher feeds improved body weight without any change in FCR (Benites et al, 2008). Bio-Mos® (Alltech Inc., Nicholasville, KY) is a yeast cell product derived from brewers yeast. A commercial YCW product (Lesaffre feed additives, Marquette-Lez-Lille, France) supplemented at 500 ppm improved FCR in corn based diets with no effect in wheat based diets in 2-week-old broilers (Morales-Lopez et al, 2010). Geier et al, (2010) reported that Bio-Mos at 0.5% (5000 ppm) in wheat based diets did not influence performance, but did alter intestinal microbiota in finishing broilers.

Very limited research is available on the effects of prebiotics on *C. perfringens* infection in broilers. In vivo studies in mammals and in vitro models revealed that FOS in diets resulted in significantly fewer *C. perfringens* bacteria in the intestinal tract (Gallaher et al, 1996; Swanson et al, 2002). In the present study, supplementation of

YCW-MOS in diets resulted in improved performance under immune stress and *C. perfringens* challenge in starting broilers. In a study by Thanissery et al, (2010) dietary supplementation of NuPro® yeast extract (Alltech Inc., Nicholasville, KY) at 2000 ppm was shown to reduce the intestinal levels of *C. perfringens* and the intestinal lesions associated with NE under *C. perfringens* challenge. On the contrary, Hofacre et al, (2003) reported that in an experimental challenge model, dietary supplementation of Bio-Mos® at 2000 ppm had no significant effect on mortality caused by NE, growth rate or FCR in 6-week-old broilers.

Immunosuppressed chickens are more likely to develop NE. Successful conditions may compromise the immune system and predispose the birds to NE (McDevit et al, 2006). Lesion scores were higher when an IBD vaccine was administered prior to challenge (Gholamiandehkordi et al, 2007). In the present study, IBD vaccine was given to birds in the challenged experiments. However, no significant differences in lesion scores were observed. YCW-MOS products act as immune modulating substances which may stimulate gut associated and systemic immunity by acting as a non-pathogenic microbial antigen, giving an adjuvant-like effect (Ferket et al, 2002). This suggests that the YCW-MOS blend may stimulate a broader immune response.

Inconsistent results were noted with regard to lowering mortality rate in the present study. In experiments one and two, YCW-MOS product reduced mortality rate compared to the control group. No differences in mortality rate were observed in the pooled data analysis from all experiments. Hooge (2004) suggested that the morality

lowering effect of MOS was its strongest attribute and this effect was significantly greater in this regard compared to antibiotic growth promoters. The findings of this research do not necessarily support this conclusion.

In a meta-analysis conducted by Hooge, (2004) it was revealed that broiler diets containing "MOS" improved final body weight by 1.75% compared to the negative control diets. It is likely that the "MOS" described in Hooge's meta-analysis not all pure mannanoligosaccharides as it was fairly common in earlier literature to describe heterogenous YCW products as MOS which is not technically correct. In 2010, The Texas State Chemist forbid the characterization of YCW additives as MOS since they are actually a mixture of many things in addition to the MOS and mannoproteins. In the current study, pooled data analysis indicated that broilers receiving the blend of 2 YCW-MOS products showed an overall improvement of 15% in body weight at 21 days of age compared to broilers receiving negative control diets. Hooge, (2004) also reported that broiler diets containing "MOS" resulted in an approximately 2% decrease in FCR at 42 days of age. This finding is also in somewhat agreement with the present study, where broiler diets supplemented with blend of YCW-MOS decreased FCR by more than 10% in 21-day-old broilers. Hooge, (2004) reported no significant differences in final body weight or FCR among broilers receiving diets supplemented with either "MOS" or antibiotic growth promoter. Hooge, (2004) also suggested that the optimal "MOS" levels in broiler diets were 0.2% (2000 ppm) from 0 to 7 days; 0.1% (1000 ppm) from 7 to 21 days and 0.05% (500 ppm) from 21 to 42 days to achieve improved body weight with

reduced FCR. However, this study suggests a blend of YCW-MOS with a concentration of approximately 250 ppm (0.025%) in starting broilers (0-21 days) is the optimum dose.

Dietary supplementation of YCW-MOS products produced growth improvement in starting broilers under challenge conditions. The YCW-MOS products may be considered as potential alternatives to traditional antibiotic growth promoters in poultry feeds.

CHAPTER IV

EFFECTS OF GUAR GUM GALACTOMANNANS WITH AND WITHOUT MANNANASE GUAR® ENZYME ON STARTING BROILER PERFORMANCE, APPARENT ILEAL ENERGY DIGESTIBILITY, INTESTINAL HISTOMORPHOLOGY AND MICROBIAL ECOLOGY

INTRODUCTION

Non Starch Polysaccharides (NSP's), naturally occurring in many foods, affect the digestion and absorption of other nutrients. NSP's are anti-nutritive compounds known to cause decreased performance of the birds by adversely affecting nutrient utilization. Addition of NSP's to broiler diets adversely affects nutrient digestibility (Choct and Annison, 1992a), which has been associated with an increase in intestinal viscosity (Choct and Annison, 1992b). The increased viscosity effectively slows down passage of digesta, increasing digesta retention time. Even though digesta retention time is increased, increased viscosity affects the action of enzymes on substrates, thus decreasing actual available nutrients (Smits and Annison, 1996). Some feed ingredients, like guar meal and copra meal, are of only limited use in poultry because of the natural occurrence of NSPs. To alleviate the antinutritive effects of NSPs, exogenous enzymes can be added to the feed.

The endosperm of guar seed consists primarily of high molecular weight polysaccharides composed of galactomannans, which are linear chains of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains. The mannose:galactose ratio is approximately 2:1 (FAO publications, 2006).

Guar gum, galactomannan NSP, is not digested in the digestive tract of monogastric animals. Guar meal, a co-product of guar gum production, is a rich protein source and can be used in animal feeds. However, use of guar meal in poultry feeding is limited by its adverse effects on feed intake, growth and production (Curl et al, 1986). Residual guar gum (galactomannan) present in guar meal is probably the major factor responsible for reported adverse effects (Verma and Mcnab, 1984; Curl et al, 1986; Lee et al, 2003).

Most studies with guar gum have concentrated on its capacity to improve glucose tolerance levels and to lower blood cholesterol levels in rats (Blackburn and Johnson, 1981; Dario-Frias et al, 1998; Favier et al, 1998) and to exhibit immunostimulatory functions in rats (Dario-Frias et al, 1998; Moriceau et al, 2000). Inclusion of 1% guar gum in piglet diets did not have any effect on histomorphology of the small intestine in piglets (Van Nevel et al, 2005).

Adding 2% guar gum to broiler diets has been reported to produce deleterious effects on broiler performance (Daskiran et al, 2004). Dietary inclusion of guar gum in poultry has been demonstrated to depress growth rate, increase intestinal viscosity associated with delayed gastric emptying, increase length and weight of intestinal tract, increase mortality rates and depress nutrient utilization (Vohra and Kratzer, 1964; Holt et al, 1979; Ray et al, 1982; Patel et al, 1982). Daskiran et al, 2004 reported that the negative effects of guar gum inclusion were alleviated by the supplementation of β-mannanase enzyme and with the enzyme, performance was equal to the control groups. Also, supplementing diets with mannanase enzyme resulted in improved feed utilization.

Partially hydrolyzed guar gum was prepared by partial hydrolysis of the mannan backbone of guar gum with β-1,4-endomannanse (Takahashi et al, 1993). Partially hydrolyzed guar gum has been reported to decrease the incidence of bacterial translocation (migration of bacteria from intestinal tract to lymph nodes) in mice (Wells et al, 1992), to promote growth of intestinal mucosal cells, to moderate diarrhea, constipation, to reduce cecal pH, to reduce *Staphylococcus* frequency and to improve the growth of cecal *Bifidobacterium* and *Lactobacillus* in rats (Takahashi et al, 1995). In a human volunteer study conducted by Tsuda et al, (1998) partially hydrolyzed guar gum has also been reported to reduce the blood glucose levels after sucrose intake. It has been reported that intact guar gum has more detrimental effects compared with partially hydrolyzed guar gum (Takahashi et al, 1995).

Partially hydrolyzed guar gum has been demonstrated to improve intestinal microflora balance in humans (Okubo et al, 1994) and in animals (Takahashi et al, 1995). With respect to poultry, Administration of partially hydrolyzed guar gum at 0.025% in young laying hen diets resulted in significant increase in *Bifidobacterium* spp. and *Lactobacillus* spp. numbers and inhibition of the Enterobacteriaceae (Ishihara et al, 2000). Interestingly, this inhibitory effect was not observed in diets containing 0.05 or 0.1% partially hydrolyzed guar gum. As *Salmonella enteritidis* belongs to Enterobacteriaceae, the authors suggested that enzyme supplementation may decrease *Salmonella enteritidis* counts.

Dietary supplementation of carob bean gum galactomannans in chicken diets reduced the populations of *Salmonella* in a challenge model, but not without adverse

effects on performance and nutrient digestibility (Vila et al, 2012). Vila et al, (2012) suggested adding β-mannanase to counteract the effects of galactomannan. Vila et al, (2012) also demonstrated that oligomeric mannoses are more efficient than monomeric D-mannose in inhibiting *Salmonella* infection in chickens. In a recent in-vitro study conducted by Badia et al, (2012), the β-galactomannan was shown to inhibit association of *Salmonella enterica* with porcine intestinal epithelial cells and to modulate immune response. D-mannose or mannose residues obtained from Yeast Cell Wall (YCW) are also known to be effective in reducing the colonization of *Salmonella* spp. (Oyofo et al, 1989a; Spring et al, 2000).

The gastrointestinal tract of chickens harbors a diversified microflora. The gut microflora acts as an efficient barrier to protect against invasion of pathogens and stimulates host defensive mechanisms. As gut microflora plays a major role in maintaining host health, altering the composition of gut microbiota in a positive way is increasingly being considered of value. Digestive microbial populations can be altered by changes in the diet. The modulation of intestinal microflora to protect host health is possible with the dietary supplementation of both probiotics and prebiotics.

To my knowledge, no study to date has evaluated the effects of guar gum galactomannans on intestinal histomorphology and microbial ecology in broilers. This study was designed to evaluate the effects of guar gum galactomannans supplemented diets with and without β-galactomannanase enzyme on starting broiler performance in terms of growth, feed efficiency, digestibility, intestinal histomorphology and microbial ecology. The hypothesis of this study is that the prebiotic properties of enzyme (β-

galactomannanase) supplemented guar gum galactomannans are equivalent to those of YCW-MOS in starting broilers.

MATERIALS AND METHODS

Experimental design and general procedure

A total of 144 newly hatched Ross 308 strain broiler chicks were purchased from Sanderson farms, Bryan, TX and randomly distributed over one Petersime battery brooder unit among four dietary treatments with six replicates of six birds per pen (24 pens, 4 treatments, 6 replicates, 6 birds per pen). A basal industrial type corn soy based broiler starter diet was prepared (Table 4-1). The basal diet was divided into 4 equally sized portions and 3 batches were supplemented with one of the three additives. Chicks were assigned to one of the following dietary treatments: 1) negative control: a basal industry type broiler starter diet, 2) positive control YCW-S: the basal diet supplemented with 500 ppm of YCW-MOS Safmannan® (LeSaffre feed additives, Milwaukie, WI) 3) GG: the basal diet supplemented with 500 ppm of guar gum galactomannans (Spectrum Chemical MFG. Corp, Gardena, CA), and 4) GGE: the basal diet supplemented with 500 ppm guar gum galactomannans along with one gram of Mannanase Guar® (AGRIaccess, Bothwell, WA). Mannanase guar® (β-galactomannanase, 1000 units/gm and cellulose 500 units/gm minimum activities) is a targeted exogenous enzyme blend designed to hydrolyze the β-galactomannan in residual guar gum of guar meal. The composition of guar gum is given in the Table 4-2.

Table 4-1: Composition and nutrient content of broiler starter diets used in this study

INGREDIENT	PERCENTAGE
Corn	58.434
Soybean meal 48%	34.493
Dl-Met 98%	0.231
Lysine HCl	0.177
AV Fat, blended	2.755
Limestone	1.561
Mono-Dicalcium Phosphate	1.537
Salt	0.512
Trace minerals premix[1]	0.050
Vitamin premix[2]	0.250
CALCULATED NUTRIENT CONTENT (%)	
Protein	22.00
ME (Kcal/Kg)	3050.00
Crude fat	5.32
Crude fiber	2.63
Calcium	0.95
AV Phosphate	0.71
Sodium	0.22
Methionine	0.56
Lysine	1.31

[1] Trace minerals premix added at this rate yields: 27.50 mg sulphur, 150 mg manganese, 16.50 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, and 0.84 mg molybdenum per kilogram diet.
[2] Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,858 IU vitamin D3, 1.47 mg menadione, 2.90 mg thiamin, 5.80 mg riboflavin, 20 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 478 mg choline, 16.50 μg Vitamin B12, 46.00 mg niacin, and 7.20 mg pyridoxine per kilogram of diet.

Table 4-2: Composition of guar gum galactomannans supplemented in the study[1]

Compound	Percent
Galactomannans	Minimum 70%
	Maximum limits
Acid insoluble matter	7.0%
Total ash	1.5%
Lead	2 mg/kg
Loss on drying	15%
Protein	10%

[1]Composition determined by Spectrum Chemical MFG. Corp (Gardena, CA).

Birds were provided *ad libitum* access to water and feed. On day 18, titanium dioxide (an indigestible marker) was added to all the experimental diets at the rate of 0.3%. Temperature in room the brooder room was thermostatic and twenty-four hour lighting was provided. Each brooder pen contained a heat lamp for supplemental heat as required. No concomitant drug therapy was used during the study. Daily observations were made with regard to general flock condition, temperature, lighting, water, feed, and unanticipated events in the house. Pens were also checked daily for mortality. Pen was the unit of measure for performance phase of this experiment. Birds dying within the first 3 days of the study were replaced. Bird weights and feed consumption (grams) by pen were recorded at day 0, 7, 14 and 21 days of the experiment.

Performance variables evaluated in this study were body weight per bird (BW), weight gain per bird (WG), total feed consumption (FC), feed conversion ratio (FCR), Productivity Index (PI), and percent mortality rate (MORT). Productivity index is calculated using the following mathematical formula:

PI = (100-MORT) x (BW/1000)/Bird age/FCR x 100

Intestinal histomorphology

On day 22, all birds were euthanized and six birds per treatment were randomly selected to collect small intestine portions. Birds were opened, small intestines were removed, and three sections throughout the small intestine were dissected. Cross-sections were excised 2-3 cm anterior to the distal end of each of the three sections – duodenum, jejunum (between the distal portion of duodenal loop and Meckel's diverticulum) and ileum (between Meckel's diverticulum and anterior portion of ileo-

caecal junction). Each cross-section was rinsed with isotonic saline (0.9% NaCl) to remove intestinal contents from the intestinal lumen, the serosal surface was labeled with indelible ink for identification purposes and fixed in 10% neutral buffered formalin. The fixed samples were embedded in paraffin and the samples were sent to the Texas Veterinary Medical Diagnostic Laboratory for the slide preparation. Slides containing intestinal sections stained with hematoxylin and eosin (H&E) were used to evaluate histomorphology of the small intestine.

Parameters measured were the height of intestinal villus and crypt depth. Histological images taken with Zeiss Axioplan microscope were examined by AxioVision LE software (Carl Zeiss Microscopy GmbH, Jena, Germany). The villus height was measured in microns from villus tip to the bottom, excluding crypt. A total of 9 measurements were taken from each intestinal region per bird thus yielding 54 observations per treatment group. The average of these 9 measurements per each intestinal region was used in statistical analysis.

Apparent ileal energy digestibility

Intestinal contents from the ileum were collected from one bird per pen yielding a total of 6 birds per treatment. All the ileal samples and two feed samples from each treatment were dried at 100^0 C for 24 h in a forced draft drying oven, cooled, finely ground and stored in a desiccator for further analysis. Gross energy content of the feed and ileal contents was calculated by bomb calorimetry in a Parr Adiabatic bomb calorimeter (Moline, Illinois). Titanium dioxide (indigestible marker) content of dietary treatments and ileal contents was measured by spectrophotometry as previously

described by Short et al, (1996). The Gross Energy (GE) values of ileal digesta were calculated using the following formula

$$\text{GE ileum digesta} = \text{GE concentration in digesta} \times \frac{\text{diet marker concentration (mg/kg)}}{\text{digesta marker concentration (mg/kg)}}$$

GE values are expressed as mg/Kcal/Kg dry matter intake.

Apparent ileal digestibility of the dietary treatments was calculated using the following formula:

$$\text{Apparent ileal GE digestibility} = \frac{\text{GE in diet-GE in ileum digesta}}{\text{GE in diet}}$$

Statistics: Data were analyzed by ANOVA using the GLM procedure with 4 treatments. Significant differences among treatment means were separated using Duncan's multiple range test at $P < 0.05$.

Intestinal microbial ecology

Distal ileal and cecal contents from 5 birds per treatment from different replicates were aseptically collected into sterile tubes containing 2.25 ml of Butterfield's buffer within 10 minutes after chickens were euthanized and contents were kept at 4^0 C until frozen and stored at -20^0 C. Ileal and cecal pH values were measured for each sample before processing them for DNA isolation. Bacterial DNA from both ileal and cecal samples were isolated from 1 ml of each sample (QIAamp Mini DNA Kit, Qiagen Inc., Valencia, CA) by following the method as described in the kit. Samples were centrifuged at 8000 x g for 10 minutes and pellets were suspended in 180 µl of TRIS EDTA Triton solution containing 20 mg/ml lysozyme (Sigma Chemical Company, St. Louis, MO) and incubated at 37^0 C for 30 min. Isolated DNA samples were checked for

quantity and quality (NanoDrop ND-1000, Thermo Scientific, Wilmington, DE) are stored at -70^0 C. Sample DNA from each treatment group (5) was pooled (50 ng each) to carry out PCR. This combined sample from several birds greatly reduces between group variation and allows for a clearer comparison of treatment differences. Amplicons were visualized by running on a 2% E-gel (Life Technologies, Grand Island, NY) for 30 min at 60 V. No PCR product or DNA was observed in any of the ileal DNA samples. These negative samples were again subjected to PCR by doubling the amount of pooled DNA (100 ng) and no ileal amplicons were noticed. So, DGGE (Denaturing Gradient Gel Electrophoresis) was carried out only with pooled cecal samples.

Bacterial diversity of ceca was investigated by performing DGGE of 16S rRNA gene PCR amplicons as described by Muyzer et al, (1993) and as modified by Hume et al, (2003). Primers (Integrated DNA Technologies, Inc., Coralville, IA) (12.5 pmole of each per reaction mixture; primer 2, 5′-ATTACCGCGGCTGCTGG-3′, and primer 3 with a 40-base G-C clamp (Sheffield et al, 1989; Muyzer et al, 1993), 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) were mixed with Jump Start Red-Taq Ready Mix (Sigma Chemical Company, St. Louis, MO) according to the kit instructions, 250 ng of pooled (50 ng for each of the 5 samples in each treatment group) template DNA and 0.1% (wt/vol) BSA to increase PCR yields, and to stabilize the enzymes (Kreader, 1996). Amplifications were carried out in a PTC-200 Peltier Thermal Cycler (MJ Research Inc., Waltham, MA) using the following program: 1) denaturation at 95^o C for 2 min; 2) subsequent denaturation at 94^{0}C for 1 min; 3) annealing at 67^0 C for 45 seconds, -0.5^0 C per cycle

(touchdown - annealing temperature is decreased by -0.5^0 C every second cycle to enrich for products containing correct matches between primers and template, and to minimize spurious priming during amplification) (Don et al, 1991) 5) repeat stets 2 to 4 for 17 cycles; 6) denaturation at 94^0 C for 1 min; 7) annealing at 58^0 C for 45 seconds; 8) extension at 72^0 C for 2 min; 9) repeat steps 6 to 8 for 12 cycles; 10) extension at 72^0 C for 30 min; 11) 4^0 C final.

Polyacrylamide gels (8% (vol/vol) acrylamide-bisacrylamide ratio 37.5:1 (Bio-Rad Laboratories, Richmond, CA.)) were cast with urea-deionized formamide gradient of 35 to 60%; 100% denaturing acrylamide was 7 M urea and 40% deionized formamide. Amplified PCR samples were mixed with an equal volume of 2x loading buffer [0.05% (wt/vol) bromophenol blue, 0.05% (wt/vol) xylene cyanol, and 70% (vol/vol) glycerol] and 5.6 µl were loades in each sample well (25-well comb; 4 mm/well). Equal volumes of 6 reference strains were mixed. Equal volumes of (3 µl) of the reference strains and 2x DGGE loading dye were mixed and added to 3 wells. Gels were placed in a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Richmond, CA.) for electrophoresis in 1x TAE (prepared from 50x stock running buffer) at 59^0 C for 17 hours at 60 V. Gels were stained in 300 ml of 1x TAE and 30 µl of SYBR Green (Sigma Chemical Company, St. Louis, MO). Relation among fragment patterns was determined using Molecular Analysis Fingerprinting Software, version 1.6 (Bio-Rad Laboratories, Herculus, CA). Band patterns in the dendrogram were analyzed by similarity coefficient percentage.

RESULTS

No significant differences were observed in production variables for any of the dietary treatments tested (Table 4-3). Broilers receiving the diets supplemented with GG exhibited significant reduction in apparent ileal energy digestibility compared to control. Broilers receiving the dietary additives YCW-S and GGE did not differ significantly in terms of apparent ileal energy digestibility values. None of the treatments showed significant difference in ileal and cecal pH values (data not shown).

Results of intestinal histomorphology are shown in Table 4-4. Broilers receiving GG in their diet had significantly reduced villus height in all the three intestinal regions and, increased crypt depth in the jejunum. This negative effect has been counterbalanced by the addition of exogenous enzyme to the diet. Broilers receiving GGE had overall intestinal villus height equal to or more than that of either the YCW-S or control group.

Table 4-3: Effect of GG and GGE on performance and apparent ileal energy digestibility of the birds at 22 days of age

Treatment	n	BW*	WG*	FC*	FCR	PI	AIED**
Control	6	776	733	1052	1.43	258	71.6[a]
YCW-S[1]	6	763	719	996	1.39	262	67.0[ab]
GG[2]	6	778	740	1028	1.39	256	56.5[b]
GGE[3]	6	792	748	1041	1.39	271	64.1[ab]
PSEM* **		19.38	20.08	24.83	0.02	9.70	3.81
P-Value		0.727	0.759	0.437	0.242	0.683	0.074

[1]Diets supplemented with 500 ppm YCW-MOS Safmannan®.
[2]Diets supplemented with 500 ppm guar gum galactomannans.
[3]GG diets containing an exogenous enzyme Mannanase Guar®.
[a,b] Means within a column with no common superscript differ significantly (P<0.05).
*Final body weight, weight gain and total feed consumption are given in grams (per bird).
**Apparent ileal energy digestibility.
***Pooled Standard Error Mean.

Table 4-4: Histomorphology of the different portions of the small intestine at 22 days of age

Treatment	n	Duodenum		Jejunum		Ileum	
		Villus height, µm	Crypt depth, µm	Villus height, µm	Crypt depth, µm	Villus height, µm	Crypt depth, µm
Control	6	767.2^{b}	50.5^{b}	699.7^{a}	59.3^{bc}	513.8^{b}	70.8^{bc}
YCW-S[1]	6	814.0^{a}	69.0^{a}	718.8^{a}	66.3^{b}	502.7^{b}	89.5^{a}
GG[2]	6	686.3^{c}	56.7^{b}	576.5^{b}	$100.^{a}$	465.7^{c}	76.5^{b}
GGE[3]	6	835.7^{a}	68.0^{a}	705.8^{a}	57.7^{c}	588.0^{a}	65.7^{c}
PSEM*		12.63	2.04	12.52	3.78	9.66	2.15
P Value <		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[1]Diets supplemented with 500 ppm YCW-MOS Safmannan®.
[2]Diets supplemented with 500 ppm guar gum galactomannans.
[3]GG diets containing an exogenous enzyme Mannanase Guar®.
[a,b,c] Means within a column with no common superscript differ significantly (P<0.05).
*Pooled Standard Error Mean.

Amplicon profiles for cecal bacteria are shown in Figure 4-1. Comparisons of cecal microbial profiles for all treatment groups resulted in two main groups with 92.6% similarity suggesting somewhat different microbial populations. Patterns from control and GG treated chicks grouped together with 94.7% similarity coefficient indicating slightly different populations between these two treatments. Microflora patterns from YCW-S and GGE treatments grouped together with 95.6% similarity coefficient. More than 95% similarity coefficient in band patterns indicates that the microbial populations examined are essentially identical. In the present study, it is revealed that the cecal microbial populations in broilers receiving either the YCW-S or GGE are identical. The cecal microbial populations from GG treated and negative control birds are somewhat similar with 94.7% relative coefficient.

Figure 4-1: Denaturing gradient gel electrophoresis gel of cecal bacterial 16S amplicon band patterns from broiler chicks receiving dietary additives at 22 days of age.
Relative similarity of band patterns is indicated by their grouping on the dendrogram and the percentage coefficient.
C1 - Ceca - Control.
C2 - Ceca - YCW-S (Diets supplemented with 500 ppm YCW-MOS).
C3 - Ceca - GG (Diets supplemented with 500 ppm guar gum galactomannans).
C4 - Ceca - GGE (GG diets containing the exogenous enzyme Mannanase Guar®).

DISCUSSION

Non-starch polysaccharides induce both physiological and morphological changes in the digestive system. NSP's increase intestinal viscosity and reduce the digestion and absorption of nutrients (Annison et al, 1995; Smits and Annison, 1996; Choct et al, 2001). It was expected that dietary guar gum would reduce the productive performance of the birds and these negative effects would be counterbalanced by the addition of the enzyme cocktail (Mannanase Guar®)to break down mannose back bone of guar gum galactomannans. However, neither dietary guar gum galactomannans alone nor with enzyme supplementation had any significant effect on the production parameters evaluated. This may be due to the low level of guar gum (500 ppm or 0.05%) tested in this investigation. Dietary guar gum is known to reduce body weight and weight gain in broilers when included at 2% level in broiler starter diets (Vohra and Kratzer, 1964; Ray et al, 1982; Daskiran et al, 2004).

Similarly, when broilers were fed diets containing 10% copra meal (containing a NSP galactomannan similar to guar gum), decreases in weight gain, feed intake and nutrient digestibility were observed (Sundu et al, 2006). Further, this decrease was proportional to the amount of copra meal in the diet. These negative effects were alleviated partially by supplementing copra meal diets with an enzyme cocktail mannanase, galactosidase, glucanase, and cellulases, thus resulting in increased weight gain, decreased FCR and improved nutrient digestibility. Galactomannans are cell wall components of most legumes (Reid, 1985) and also found in soybean in small quantities (Ward and Fodge, 1996). Therefore, appropriate exogenous enzyme supplementation

may improve nutrient utilization in regular corn soy based diets (Daskiran et al, 2004; Zou et al, 2006) as well as other diets not necessarily based on soybean meal

Broilers receiving GG diets exhibited decreased apparent ileal digestible energy and enzyme supplemented diets did not show an improvement in apparent ileal digestible energy. No significant difference in apparent ileal energy digestibility was observed among broilers receiving YCW-S and GGE diets. Daskiran et al, (2004), reported that the broiler diets containing 1% and 2% guar gum produced a significant reduction in dietary metabolizable energy and net energy gain, and supplementation of guar gum diets with 0.05% β-D-mannanase partially reduced those negative effects. The viscous nature of guar gum is responsible for reduced digestibility and nutrient absorption, which occurs by delayed gastric emptying, impairing the action of digestive enzymes and reducing the contact of nutrients with the absorptive surface (Read, 1986).

Geier et al (2009) did not observe any significant differences in apparent metabolizable energy and ileal digestible energy among tested dietary treatments, control, AGP (Zinc bacitracin 50 ppm), MOS (Bio-Mos, Alltech Inc., Nicholasville, KY) at 5 g per kg and FOS (Fibrulose F97,Cosucra Group, Warcoing, Warcoing, Belgium) at 5 g per kg (5000 ppm) of diet. The dietary addition of MOS did not affect the digestibility of starch, protein and fat in 3 week old broilers (Yang et al, 2008). In the present study, apparent ileal energy digestibility of broilers receiving 500 ppm YCW-S was not significantly different from the negative control.

The results of the current study indicated that broilers receiving GG diets exhibited reduced intestinal villus height whereas broilers receiving GGE diets showed

increased intestinal villus height. Increased intestinal villus height offers a greater surface area for absorption and should improve nutrient utilization. Dietary inclusion of MOS is believed to improve gut health by increasing villus height, uniformity and intestinal integrity (Santin et al, 2001; Loddi et al, 2002; Zhang et al, 2005; Baurahoo et al, 2007). In this study, the YCW-S treated broilers showed significant improvement in duodenal villus height, with no effect in jejunum or ileum. However, GGE treated group displayed intestinal villus height equal to or better than positive control YCW-S treated birds.

In this investigation, no significant differences were observed in the ileal pH and cecal pH values among any of the dietary treatments. Yang et al, (2008) did not observe any difference in cecal pH of YCW treated birds, but ileal pH values were significantly higher compared to the control when wheat barley diets were fed. The inconsistency in the results of various reports in the literature may be due to the differences in nutrient composition of diets, rearing conditions and other environmental factors.

The PCR-based DGGE technique is useful for determining the microbial community shifts induced by various dietary treatments (Hume et al, 2003). Microbial ecology results of the present study indicated that the cecal microbial populations from the positive control (YCW-S) and GGE diets grouped together with a 95.6% relative similarity coefficient suggesting identical microbial populations in these two treatment groups. Dietary YCW are known to reduce pathogenic bacterial load and improve beneficial bacterial colonization in broilers (Spring et al, 2000; Baurhoo et al, 2007). Very similar microbial patterns were observed in YCW-S and GGE treated groups.

Therefore, enzyme supplemented guar gum galactomannans may have a beneficial effect on host health. The microbial shifts in the cecum observed in the present study did not have any effect on the growth rate and feed conversion ratio. This finding is in consistent with the results reported by Geier et al, (2009) where significant overall intestinal (combined jejunum, ileum and cecum microbial populations) microbial shifts observed with the feeding of YCW (Bio-Mos, Alltech Inc., Nicholasville, KY) did not result in improved performance in broilers. Intestinal microbial communities can modify energy metabolism by exhibiting a buffering or a counter-productive action on the utilization of energy in chickens (Muramatsu et al, 1994).

GGE treated birds exhibited intestinal function equal to the positive control birds, and, microbial patterns from these two groups are identical, whereas GG treated birds poorly performed compared to the negative control. Therefore, GGE may have a positive effect on intestinal microbiota balance and potentially effective in preventing on some pathogenic microbial colonization. The results indicated β-galactomannanase supplemented as Mannanase Guar® may be effective in alleviating some negative effects associated with the feeding of guar meal.

CHAPTER V

SUMMARY AND CONCLUSIONS

Previous researchers have described YCW products as "MOS" in their reports, which is not technically correct. YCW products are a rich source of MOS as mannoproteins but YCW also contains high concentrations of β-glucans. The first study (chapter 3) of this research evaluated the efficacy of yeast cell wall products containing mannanoliogsaccharides on starting broiler performance. *Clostridium perfringens* infection was experimentally induced in broiler chicks under immune suppression to better assess the prebiotic potential of these feed additives. The second study (chapter 4) evaluated the prebiotic efficacy of guar gum galactomannans supplemented with an exogenous β-galactomannanase enzyme (Mannanase Guar®) in starting broilers.

YCW-MOS supplemented diets resulted in improved performance with a decrease in FCR. Both of the YCW-MOS products obtained from two sources (Safmannn® – bakers yeast; Pronady® – brewers yeast) produced a significant improvement in body weight with reduced FCR compared to the control birds. However, no difference between the two sources of the YCW-MOS additives was detected. Interestingly, a blend of these two products appears to be the best YCW-MOS to promote growth rate. Broilers (3-week old) receiving the blend of YCW-MOS products in their diets showed an overall improvement of 15% in body weight, and 10% reduction in FCR with higher productivity index compared to the control birds not receiving any YCW-MOS product.

Broilers treated with 125, 250, 375 or 500 ppm of YCW-MOS additive exhibited lower FCR compared to the control birds. Broilers receiving 250 ppm YCW-MOS showed a peak response in body weight when body weight was fitted to dose in second order regression line. YCW-MOS additive at 375 ppm produced a higher productivity index. This investigation also suggests that the optimal level of YCW-MOS products (both brewers and bakers yeast) as growth promoting feed additives in starting broiler diets is approximately 250 ppm.

These findings are commercially important as previous literature suggested an optimal dose of more than 500 ppm or 1000 ppm YCW-MOS additive in starting broilers. This is the first kind of investigation which evaluated different sources and a blend of YCW-MOS products. Supplementation of YCW-MOS additives in broiler starter diets improved both growth rate and feed efficiency. Based on the results of this investigation, LeSaffre feed additives (Milwaukie, WI) has developed a new product called CWall® (Appendix 4) to improve growth rate in poultry. YCW-MOS products may be considered as potential alternatives to traditional antibiotic growth promoters in poultry feeds.

The presence of non-digestible carbohydrates in poultry diets influences nutrient utilization. Galactomannan, a non-starch polysaccharide naturally occurring in several plant legumes is known to depress nutrient utilization by increasing the viscosity of intestinal contents. Adding appropriate exogenous enzymes to reduce the negative effects of some NSPs is a common practice in poultry feeding. These enzymes improve digestibility of the polysaccharides, otherwise not digested by the host system.

Galactomannan gum obtained from the plant legume guar may be considered for prebiotic functions.

In the second study (chapter 4) of this research, prebiotic efficacy of guar gum galactomannans supplemented with an exogenous β-galactomannanase enzyme (Mannanase Guar®) was evaluated in starting broilers. This investigation indicated that neither guar gum nor enzyme had any effect on the production parameters. The guar gum galactomannans (GG) inclusion significantly reduced apparent ileal energy digestibility values compared to the control. Mannanase Guar® supplementation did not significantly affect apparent ileal energy digestibility.

Broilers treated with GG diets showed a reduction in intestinal villus height compared to the negative control. Broilers receiving the positive control YCW Safmannan® and enzyme supplemented GG showed overall improvement in intestinal function as evident by increased villus height and reduced crypt depth. Increased villus height is thought to offer a greater surface area for nutrient absorption thus resulting in increased nutrient utilization.

Comparison of the cecal microbial profiles for all treatment groups resulted in two main groups with 92.6% similarity. Band patterns from control and guar gum treated chicks grouped together with a 94.7% similarity coefficient and microflora patterns from YCW Safmannan and enzyme supplemented GG treatments grouped together with a 95.6% similarity coefficient. The cecal microbial populations from Mannanase Guar® supplemented GG diets and the positive control YCW Safmannan supplemented diets were essentially identical.

YCW-MOS additives are known to reduce pathogenic bacterial load and improve beneficial bacterial colonization in broilers. Identical microbial patterns were observed in YCW and enzyme treated groups in this study. Therefore, enzyme supplemented GG may have a beneficial effect on host health.

Mannanase Guar® supplemented GG diets exhibited prebiotic properties by improving intestinal function and altering intestinal microbiota. The presence of residual guar gum in guar meal is responsible for limited use of protein rich guar meal in poultry diets. The enzyme cocktail (Mannanase Guar®) evaluated in this study may be effective in reducing the detrimental effects associated with guar gum inclusion in poultry diets suggesting a potentially cost effective alternative to traditionally used growth promoting antibiotics. Guar gum is also generally known to improve pellet quality suggesting a possible dual benefit in pelleted poultry diets, when used in combination with an appropriate exogenous enzyme to reduce the negative viscosity effect of this non starch polysaccharide.

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APPENDIX

SAFMANNAN®

A Yeast-derived source of Mannanoligosaccharides

PRODUCT CODE:45335

PRODUCT INFORMATION SHEET

Description: Safmannan is a source of Mannanoligosaccharides (MOS) derived from primary inactivated yeast (*Saccharomyces cerevisiae*) for use in rations for poultry, swine, cattle, and fish.

Ingredient Statement: Dried yeast cell wall

Chemical Composition:

Moisture	2-3%
Dry Matter	97-98%
Proteins	14-17%
Fat	17-23%
Ash	3-5%
Mannans	22-24%
Beta-glucans, total	24-26%

Physical Composition:

Appearance	Light tan powder
Aroma	Mild yeasty
Flavor	Mild yeasty, slight metallic note
Mouthfeel	Chalky, drying

Packaging: 25 kg (55 lb) poly-lined kraft bag

SAFMANNAN® CODE 45335

Approximate analysis of vitamins, minerals, and amino acids (essential amino acids are underlined).

Vitamins	Mg/100g	Minerals	Mg/100g	Amino Acids	Mg/g
Vitamin A	100 IU	Calcium	154	Alanine	8.2
Niacin	2.13	Copper	8	Ammonia	2.6
Riboflavin (B_2)	0.1	Iron	4.6	Arginine	5.9
		Phosphorus	862	Aspartic Acid	9.9
		Potassium	377	Glutamic Acid	13.8
		Sodium	59	Glycine	5.0
				Histidine	2.7
				Isoleucine	5.5
				Leucine	9.1
				Lysine	9.0
				Methionine	1.1
				Phenylalanine	4.9
				Proline	4.3
				Serine	7.7
				Threonine	9.0
				Tyrosine	4.1
				Valine	6.1

Fatty Acid Composition

C16 (palmitic acid)	8-12%
C16-1 (palmitoleic acid)	40-50%
C18 (stearic acid)	1-4%
C18-1 (oleic acid)	30-40%
C18-2 (linoleic acid)	3-5%
C18-3 (linolenic acid)	1-3%

Phospholipid Composition

Phosphatidyl choline	40-50%
Phosphatidyl inositol	15-30%
Phosphatidylserine	7-10%
Phosphatidylethanolamine	5-6%
Lisophosphatidylcholine	0-13%

Specification	**Guarantee**	**Typical Analysis**
Total Bacteria Mold	2000/g maximum	<10 - 100/g
Coliform E. coli Salmonella	10/g maximum	<10/g
	< 10 CFU/g Neg /10g Neg/1500g	< 10 CFU/g Neg / 10g Neg/1500g

Storage: Store under cool, dry storage conditions. The product is stable for 48 Months.

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Pronady 500™

Product Information Sheet

General Description

Pronady 500 is a source of Mannanoligosaccharides (MOS) derived from inactivated brewer's yeast (*Saccharomyces cerevisiae*) for use in rations for poultry and young livestock.

Chemical Composition

Moisture	3-5%
Dry matter	95-97%
Proteins	32-40%
Fats	3-7%
Phosphorus	1-2%
Mannans	12-16%
Beta-glucans, total	24-28%
Ash	3-5%

Physical Composition

Color	Cream to tan
Smell	Typical of yeast, beer
Impurities and defects	No evidence of extraneous or foreign material

Microbiological Composition

Coliforms per gram	negative
Salmonella	negative
Staphylococcus	< 1

Typical Usage Level

1 to 2 pounds per ton of feed.
500 to 1,000 grams per metric ton of feed.

Packaging

Fifty-five pound net weight multi-wall Kraft bags with polyethylene liner.

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BIOSAF®

Product Information Sheet

General Description

BIOSAF® is a concentrate of live *Saccharomyces cerevisiae* yeast cells. This strain (SC47) is carefully grown to yield maximum uniformity and consistency. BIOSAF is designed to be incorporated in pelleted feeds due to a unique natural coating technology that provides excellent resistance to heat shock during the pelleting process. Each batch produced is thoroughly tested to insure conformity to microbiological, physical, and chemical standards.

Chemical Composition

Moisture	6.5-8.5%
Dry material	92-94%
Proteins	40-46%
Fats	4-7%
Carbohydrates	40-50%
Ash	5-7%

Physical Composition

Color Smell Particle size	Tan
Impurities and defects	Typical of yeast Granularsphere: 2-3 mm. No evidence of extraneous or foreign material

Microbiological Composition

Live yeast cell count per gram	10 Billion CFU minimum
Coliforms per gram	Negative
Salmonella	Negative in 25 g of BIOSAF

Typical Usage Level

0.1-0.3 percent of the total daily ration.

Packaging

BIOSAF® is sealed in 25-kilogram, multi-wall polyethylene-lined kraft bags to preserve activity and prevent loss of product during shipping and storage.

C Wall®

A Yeast-derived source of Mannanoligosaccharides

Product Code ------

Product Information Sheet

General Description: C Wall is a source of Mannanoligosaccharides (MOS) derived from primary inactivated yeast (*Saccharomyces cerevisiae*) for use in rations for poultry, swine, cattle, fish, and companion animals.

Ingredient Statement: Yeast extract

Chemical Composition:

Moisture	3-5%
Dry Matter	95-97%
Proteins	26-32%
Fat	10-16%
Ash	8-12%
Mannans	17-22%
Beta-glucans, total	20-32%

Physical Composition:

Appearance	Light tan to tan powder
Aroma	Mild yeasty
Flavor	Mild yeasty, slight metallic note
Mouthfeel	Chalky, drying

Packaging: 25 kg (55 lb) poly-lined kraft bag

Storage: Store under cool, dry conditions. This product is stable for 48 months.