THE EVALUATION OF VARIOUS ANTIBIOTIC ALTERNATIVES FOR OPTIMUM POULTRY PERFORMANCE UNDER PATHOGEN CHALLENGE

A Dissertation

by

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ABSTRACT

This research was conducted to evaluate the effect of different types of feed additives (probiotics, prebiotics, postbiotics and their mixtures) on challenged or Noneee-challenged broilers with *Clostridium perfringens* or *Salmonella Typhimurium*, in starter or full-term chickens' performance.

In the frirst study, the objective was to determine the effect of adding postbiotics (XPC® and XPC-Ultra®) and prebiotics (Safmannan® 125 and Safmannan® 250) to broiler chickens' performance while challenging with *Salmonella Typhimurium*. The results of this study did not show significant enhancement in broilers' performance at day 10. Safmannan® 125 showed significant reduction in *Salmonella Typhimurium* count on the plates compared to all other challenged groups.

In the second study, postbiotics (XPC® and XPC-Ultra®) and prebiotics (Safmannan® 250 and Safmannan® 500) were used to study their effects on broiler chickens performance while challenging with *Clostridium perfringens.* The results showed these additives groups did not retain the performances parameters to Noneee-challenged control-like at day 21, as they did not enhance performance significantly compared to challenged control group with no additives.

In the third study, the objective was to evaluate the effect of adding Phelio Microsaf® probiotic, Envera Goplus® probiotic, Safmannan® prebiotic, and combination of Envera Goplus® + Safmannan® on broiler performance while challenging with *Clostridium perfringens.* The results showed no significant differences in performance

parameters between the additives groups and challenged control group at day 21 while challenging with *Clostridium perfringens.*

In the fourth study, the objective was to evaluate the effect Envera Goplus® probiotic, Actisaf® probiotic, Safmannan® prebiotic, and XPC® postbiotic on full term broiler chickens' performance, fecal dry matter percentage, and intestinal morphology of the chickens. The results showed there were no significant enhancement of full term broilers' performance at day 42 and no differences in fecal dry matter percentage or intestinal morphology between groups were found.

In the final study, the objective was to evaluate the effect of different concentrations of Safmannan® prebiotic + *Bacillus* probiotic on broilers' performance while challenging with *Clostridium perfringens.* The results showed no significant enhancement in broilers' performance while adding the combinations of Safmannan® and *Bacillus.*

In conclusion, adding the mentioned additives in these concentrations did not enhance broilers' performance while challenging with or without pathogens.

DEDICATION

I dedicate my dissertation to…..

To my beloved and only sister, Nadine: for all her love, help and sacrifice to allow me to write and finish this chapter of my life successfully and by taking care of my little sons. For her patience, sweetness, and kindness, I love you and owe you forever.

To my two lovely and sweet boys: for their love and care which gave me the hope in this life to live and continue my journey, and to be more productive and energized, and find understanding in life. You are my two wings that raise me to the skies.

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To my grandmothers' soul: for her prayers that I know she keeps sending to me for survival and happiness. You are the missing piece of my heart that I hope to meet again one day.

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NOMENCLATURE

YCW	Yeast Cell wall
MOS	Mannanoligosaccharides
FCR	Feed conversion ratio
PI	Productivity index
VH	Villi height
CD	Crypt depth
VH/CD	Villi height / crypt depth

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

From 1961 to 2019, annual poultry production improved from 9 to 132 million tons (FAO, 2021). The poultry industry is estimated to grow 2–3% annually between 2015 and 2030, which is the highest growth rate in the livestock sector (FAO, 2015). Many antibiotics have been used as growth promoters in animal farms. Due to using antibiotics, animal growth improved, and a reduction of morbidity and mortality in animals occurred because of clinical and subclinical disease suppression (George and Fagerber, 1984).

Thenceforth, antimicrobial resistance has been a public health threat caused by appropriate and inappropriate use of anti-infective medicines for human and animal health and food production. Antimicrobial resistance is posing a potential threat to human health (WHO, 2020). As a result, dietary antibiotics have been used in commercial poultry production to improve growth performance and control infectious diseases (Gadde *et al.* 2017). Numerous experiments are researching the effect of using antibiotics in poultry feed because of rising consumer awareness about antibiotic resistance and demanding antibiotic-free animal products (FDA, 2013). The Federal Department of Agriculture (FDA) has instituted a major change in the medical importance of antibiotics which can be legally used in feed and water for animal production. The FDA has eliminated the use of drugs for production which enhance

growth and feed efficiency. Currently, antibiotics are only used as therapeutic drugs under supervision of licensed veterinarians to ensure the correct usage of antimicrobial antibiotics, which are important in food producing animals (FDA, 2022). In the countries which stopped using antibiotic growth promoters in the poultry industry, the rate of necrotic enteritis associated with *Clostridium perfringens* has increased (Van Immerseel *et al.*, 2009). Therefore, the quest for antibiotic alternative products to improve poultry productivity and prevent diseases has increased (Gadde *et al.*, 2017). Probiotics, prebiotics, their mixtures (symbiotic), and postbiotics are some of the several classes of antibiotic alternatives which have been recommended in poultry production. Probiotics are defined as mono or mixed cultures of live organisms which should be administered in sufficient amounts to lead to benefiting the health of the host (FAW/WHO, 2002). *Bacillus* and *Saccharomyces cerevisiae* are beneficial microorganisms which have been tested as probiotics in poultry in the past (Kabir, 2009). Using prebiotics is considered an alternative methodology to sub-therapeutic antibiotics in livestock to reduce enteric diseases in poultry due to its effectiveness in enhancing specific bacterial populations, which plays an important role in enteric disease reduction. Prebiotics, a term first introduced by Gibson and Roberfroid, are Nonee-viable feed components that modulate the microbiota which reflect health benefits on the host (FAO, 2006). Some oligosaccharides have been considered as prebiotics such as mannan-oligosaccharide (MOS), fructo-oligosaccharide (FOS), insulin, and some other Nonee-starch polysaccharides (Patterson and Burkholder, 2003; and Steiner, 2006). Mannan-oligosaccharide (MOS) is an oligosaccharide derived from the outer cell-wall layer of

the yeast *Saccharomyces cerevisiae*, which has been studied as a prebiotic additive in poultry diets. Results show a significant increase in body weight and feed conversion efficiency has been improved in broiler chickens through MOS addition in different levels to their diets (Abdaljaleel, 2018, and Benites *et al.,* 2008). MOS supplementation to poultry showed improvement in immune-competence in the intestine (Shanmugasundaram & Selvaraj, 2013) and increased intestinal villi height (Yang *et al.,* 2007). Some of *Bacillus spp.* could be used as probiotic or antibiotic alternative additive, as research showed that adding *Bacillus licheniformis* to the broiler chicken diet, which challenged with *Clostridium perfringens,* increased weight gain compared to Nonee-medicated group (Knap *et al.,* 2010). As well as feeding *Bacillus amyloliquefaciens* as a probiotic to broiler chickens showed positive linear effect on body weight and negative linear effect on feed conversion ratio of the chickens (Hong *et al.,* 2019 & Ahmed *et al.,* 2014). *Bacillus pumilus* secrete antimicrobial substance (Hasan *et al.,* 2009), which serves as infection preventative is poultry feed.

The specific objectives for this research are:

1. Evaluation of the effect of Phileo® Safmannan® prebiotic and Diamond V® yeast culture postbiotic (XPC® and XPC Ultra®) on the performance of broiler chickens when challenged with *Clostridium perfringens* or exposed to *Salmonella Typhymurium*.
2. Evaluation the effect of Microsaf®, Envera Goplus® probiotics, Safmannan® prebiotic on starter broiler performance in birds subjected to bursa vaccine and *Clostridium perfringens* challenge.

3. Evaluation the effect of Safmannan ® prebiotic, Actisaf®, Envera Goplus® probiotics, and XPC® postbiotic on full term broiler performance.
4. Evaluation of the mixture of different levels of Safmannan® prebiotic and *Bacillus* probiotic on birds subjected to bursa vaccine and *Clostridium perfringens* challenge.

LITERATURE REVIEW

Antibiotic Resistance

Antibiotic resistance is defined as the ability of a microorganism to resist the killing effects of an antimicrobial agent (Reygaert, 2018). The development and the spread of antibiotic resistance is a cause for concern for consumers after increasing multi-drug resistant bacteria. It is acknowledged that more than 60% of all antibiotics that are produced globally find their use in animal production for therapeutic or Nonee-therapeutic uses. For this reason, the use of antibiotics in animal production has been linked to the development and spread of resistant bacteria (Kasimanickam *et al.*, 2021).

Antibiotic Alternatives

Searching for alternative for the antibiotics is the outcome of increasing regulations regarding the use of antibiotics as growth promoters in animal feed. Increasing consumer demand for poultry products produced with no antibiotics has led researchers to find alternatives to antibiotics as well (Gadde *et. al.*, 2017). The ultimate antibiotic alternative should have the same effect as antibiotics on animal performance (Huyghebaert *et al.*, 2011). Many antibiotic alternatives have been tested in poultry

production. Probiotics, prebiotics, and symbiotics are considered antibiotic alternatives (Gadde *et al.*, 2017). Postbiotic is a new type of feed additives that could be used as antibiotic alternative that works like growth promoter and anti-stress treatment in poultry plants (Humam *et al.,* 2019).

Probiotic

Probiotics are live microorganisms that are supplied as feed additives to improve intestinal balance, which enhance the health of the host animal. Probiotics should be administered in adequate amount to give the desired benefits (FAO/WHO, 2002). Alagawany *et al.*, (2018) stated that using probiotics in poultry diet can limit various infectious diseases, and the optimal effects of probiotics can occur through appropriate selection of probiotic strains which are used as feed additives.

Research results showed that adding probiotics to broiler chicken's diets could improve production. Feeding probiotics to poultry could lead to intestinal health improvement by supporting the beneficial microbial populations and suppressing harmful bacterial growth in the digestive tract (Jadhav *et al.*, 2015). The benefits of probiotics can occur directly in the gastrointestinal tract and indirectly through modulation of immune response in poultry (Krysiak *et al.,* 2021). Additionally, feeding probiotics to broiler chicken enhances utilization of proteins and increases feed conversion ratio. Adding probiotic to broiler chicken's diets showed decreases in diarrhea cases and mortality and an increase in body weight (Jadhav *et al.*, 2015).

Mode of action of probiotics:

Using probiotics to improve poultry production through inhibiting pathogens and enhancing nutrient absorption through several mechanisms, such as: producing antibacterial substances and organic acids such as hydrogen peroxide and bacteriocin (Tiwari *et al.*, 2012), competitive inhibition of pathogens by blocking of pathogenic bacterial adhesion sites to intestinal epithelial binding sites, as well as competition for nutrients (Tiwari *et al.* 2012), and modulating host immune response by specific mechanisms, which include: impacting regulatory T cells, antigen presenting cells, effector T and B cells, and enterocytes (Oelschlaeger, 2010). Probiotics can also regulate the production of anti- and pro-inflammatory cytokine (Roselli *et al.*, 2005). Probiotics can stimulate the production of antibodies (sIgA), enhance natural killer and macrophages cells activity, and modulate dendritic cell's function (Tiwari *et al.,* 2012). Immunomodulation property of probiotic organisms is exerted through microorganisms' effect on T helper cells in a strain-specific manner. Furthermore, probiotics can activate various immune cells (Fong *et al.*, 2016). Probiotics can also stimulate the intestine to regenerate the intestinal mucosa (Perdigon *et al.,* 1995). Probiotics helps proper digestion by improving digestive enzymes secretion, and could stimulate function of epithelial barrier by regulating mucous production and motility of intestine. Probiotics stimulate acidic pH, which enhances absorption of proteins and minerals like copper, calcium, iron, manganese, and magnesium (Raghuwanshi *et al.*, 2015).

Types of Probiotics Used in Broiler Chickens Production

Multiple bacterial species have been tested and used as probiotics in poultry, including broiler chickens, such as; *Bacillus, Bifidobacterium, Enterococcus, LactoBacillus, Lactococcus spp.* and *Streptococcus*. Yeast, such as some of *Saccharomyces spp.*, have been used as probiotics in the past (Kabir, 2009). *Saccharomyces cerevisiae* is used in broiler chicken production as antibiotic replacement, which could be used as a growth promoter in healthy and disease challenged birds (Ahiwe *et al.*, 2021). Adding *Saccharomyces cerevisiae* to broiler chicken's diets enhanced weight gain, feed efficiency, serum immunoglobulin A, and immunoglobulin G, while also lowering blood urea concentration. Adding *Saccharomyces cerevisiae* as a probiotic to broiler chicken's diets improved nutritional properties safely (Sun *et al.,* 2019).

Bacillus spp.

Bacillus species are rod-shaped bacteria, which are aerobic or electively anaerobic. They are Gram-positive bacteria, but some species may turn to Gram-negative in aged cultures, which are endospore forming. These spores are resistant to cold, heat, desiccation, disinfectants, and radiation. The numerous species of *Bacillus* demonstrate the many physiologic capabilities which allow them to survive in natural environments (Turnbull, 1996). Some physiological characteristics make *Bacillus spp.* safe to add to a poultry diet, which helps keep the bacteria active. Many strains of *Bacillus spp.* are used in poultry production as probiotics widely. The wide use of this bacteria is because they tolerate high temperatures and acidic pH as studies have shown.

These feed additives reach the intestine without damage because of the low stomach pH and high body temperature of the chicken. These properties enhance the quality of the probiotic in addition to the health benefits behind using it (Patlan *et al.*, 2019).

Bacillus amyloliquefaciens

Bacillus amyloliquefaciens is a species of *Bacillus* bacteria that synthesizes a natural antibiotic protein barnase (bacterial RiboNucleASE), they are also reported to produce various enzymes including α-amylase, protease, lipase, cellulase, xylanase, pectinase, aminotransferase, peroxidase, and laccase (Ngalimat *et al.*, 2021). *Bacillus amyloliquefaciens* produce antimicrobial compounds that capable to inhibit pathogens' growth like Nonee-ribosomal peptides and polyketides (Ngalimat *et al.*, 2021). Polyketides are secondary metabolites which can show verities bioactivities like antibacterial, antifungal, and anticancer. *Bacillus amyloliquefaciens* could be used as antiviral, immune-suppressant, and anti-inflammatory activity agent (Risdian *et al.*, 2019). *Bacillus amyloliquefaciens* is also known for producing a-amylase and protease (Priest *et al.*, 1987).

In a recent research study, scientists found that spores of *Bacillus amyloliquefaciens* can tolerate very high dry heat temperatures at 420 °C (877 °F). The DNA was able to replicate after exposure to this high heat temperature, due to amylases and proteases enzymes which were active after heat treated spores regenerated directly (Beladjal *et al.*, 2018). In poultry, *Bacillus amyloliquefaciens* is a species used as probiotic in poultry for its beneficial characteristics. Adding *Bacillus amyloliquefaciens* significantly enhanced the growth performance, carcass quality, immunity, and serum

biochemicals of broiler chickens (Ahmat *et al.*, 2021). In another study, feeding *Bacillus amyloliquefaciens* as a probiotic to broiler chickens showed positive effects on body weight and negative effects on feed conversion ratio of the chickens (Hong *et al.*, 2019 & Ahmed *et al.*, 2014). Feeding *Bacillus amyloliquefaciens* to broiler chickens enhanced growth performance. That enhancement was a result of improving of cecal microflora, intestinal morphology, and better nutrient utilization (Lei *et al.*, 2015). *Bacillus amyloliquefaciens* as a feed additive lead to feed conversion ratio (FCR) improvement in challenged broiler with both of *Eimeria maxima* and *Clostridium perfringens* pathogens significantly as the FCR were 1.664 and 1.704, while the mortality percentages were 4.167 and 5. 730 respectively, in comparing feed additive versus no feed additive groups (Oliveira *et al.*, 2019). In another study, feeding *Bacillus amyloliquefaciens* to broiler chickens raised the body weight gain and decreased the feed conversion ratio significantly compared to the control group. Villi height to crypt depth were all significantly higher in the probiotic groups compared to the control group (Lei *et al.*, 2015).

Bacillus pumilus

Bacillus pumilus is used in poultry production as a feed additive. Probiotics containing *Bacillus pumilus* were capable of enhancing the maturity of the cecal microbiota (like; *Ruminococcaceae*, *LactoBacillus*, and *Bifidobacterium*) earlier in life, which has an impact on poultry performance due to health promoting effects of these microorganisms (Bilal *et al.*, 2021). Supplementation of *Bacillus pumilus* protease to broiler chickens reduced the final cost of the production as a result of improving feed

intake and digestibility (Pudova *et al.,* 2020). The weight gain at five weeks of the animal's age were 1528.7 g in the additive group compared to 1465.9 g in the control group with a significant difference at 0.004 p-value and the feed conversion ratio enhanced significantly, reported as 1.61 and 1.75 respectively, in the additive and control groups (Pudova *et al.,* 2020). Digestibility coefficients of protein in the additive group were enhanced compared to control group, and they were 81.1 and 74 respectively (Pudova *et al.,* 2020). Bilal *et al.,* (2020) found in a study that adding *Bacillus pumilus* to the broiler chickens' diet led to a significant body weight and feed intake enhancement at day 42 compared to control group, which their body weights were 3,033 g and 2,780 and the feed intake were 214 g and 2206 g in the additive and control groups respectively. *Bacillus pumilus* bacteria secretes a antimicrobial substance, which could be used as an antibiotic alternative (Hasan *et al.,* 2009). These antimicrobial substances could have inhibitory effects against some pathogens, such as: *Salmonella gallinarum, Salmonella enterica* ATCC13076, and Chicken *Escherichia coli* O78 (Chu *et al.*, 2019).

Bacillus licheniformis

Bacillus licheniformis has been widely used in the poultry industry. This bacteria can be used as antibiotic alternative to improve growth performance in poultry (Liu *et al.,* 2012). *Bacillus licheniformis* could induce microphage extracellular traps, which are fundamental in the elimination of microbial pathogens (Romo-Barrera *et al.,* 2021). Supplying *Bacillus licheniformis* to broiler chickens showed significant enhancement in the average daily weight gain in both cocks and hens compared to control groups without additive. Significant enhancement of feed conversion ratio occurred in groups

supplied with *Bacillus licheniformis*, compared to the control group (Liu *et al.*, 2012). *Bacillus licheniformis* could be used as alternative treatment to treat necrotic enteritis caused by *Clostridium perfringens* in the poultry industry (Knap *et al.*, 2010).

Lactobacilli

Lactobacilli species are microaerophilic gram-positive bacteria which are found in milk, fruits, and soil. Lactobacilli could support intestinal health of chickens by balancing intestinal microflora (Chen *et al.*, 2017; Chen *et al.*, 2005; and Lan *et al.*, 2003). *LactoBacillus* culture showed enhanced effects on broiler chicken performance as it enhanced feed conversion ratio (FCR) compared to control group at day 21. The values of FCR were 1.39 and 1.53 respectively in *LactoBacillus* and control groups (Chen *et al.*, 2017). In another study, adding *LactoBacillus reuteri* to broiler diet enhanced FCR in week 5, 6, 7, and 8 of the chickens age (Bhogoju *et al.*, 2021). Adding *Lactobacilli* probiotics to broiler chicken's diets led to improvement in body weight and weight gain according to a study performed by Fesseha *et al.*, (2021). The beneficial effects of adding *LactoBacillus sp.* to broiler chicken's diets could occur by enhancing intestinal villi permeability, therefore improvement of nutrients absorption occurs which lead to body weight enhancement (Pertiwi and Mahendra, 2021). *Lactobacilli* can be used as antibiotic alternative, since it has multiple mechanisms to inhibit pathogens like producing organic acids, producing hydrogen peroxide (H2O2), and producing bacteriocin (Taheri *et al.*, 2009). Adding a combination of *LactoBacillus* and yeast probiotics to broiler chicken's diets enhanced intestine morphology by increasing villi

height in jejunum and increasing Villi: Crypt ratio at day 21 and 42 compared to the control group. Additionally, adding this combination to broiler chicken's diets enhanced the crypt depth at day 21 but not at day 42 (Qiu *et al.,* 2022). *LactoBacillus plantarum* showed improvement in animal feed intake and weight in broiler chickens (Benbara *et al.,* 2020).

Saccharomyces cerevisiae

Saccharomyces cerevisiae yeast is a unicellular fungus which multiplies by budding or fission. The large diameter sized cells are 5– 10μm and the small diameter cells are 5 μm. The cells of *Saccharomyces cerevisiae* are pigmented and usually known as brewer's or baker's yeast, which forms cream color colonies when grow on a surface and generally ellipsoid in shape cells (Walker and White, 2018). *Saccharomyces cerevisiae* produces nutrients such as amino acids and enzymes, including amylase, glucanases, lipase, mannanases, and protease, and produces vitamins (Klis *et al.,* 2002). *Saccharomyces cerevisiae* is used to produce ß-glucan and Mannan-oligosaccharide, which is used as prebiotic and has therapeutic applications (Kim *et al.,* 2007), and ß-glucan could play role as an immune modulating factor (Steenwijik *et al.,* 2021).

Dietary supplements containing *Saccharomyces cerevisiae* improves growth performance. This kind of supplementation improves immune functions as well as digestibility of calcium and phosphorus. Intestinal mucosal morphology of broiler chickens could be enhanced by adding yeast culture to the diet as well (Gao *et al.,* 2008). *Saccharomyces cerevisiae* probiotic and prebiotic products could be used as antibiotic alternatives and growth promoters in broiler chicken production (Ahiwe *et al.,* 2021).

The cell wall matrix of *Saccharomyces cerevisiae* consists of 40% manno-proteins of the dry mass. Mannan alone, form 31% of the dry mass of the yeast. Mannan structures carry several proteins which help in molecular recognition and adhesion (Klis *et al.,* 2002). Adding yeast culture to the broiler chicken diet at 2.5 g/kg enhanced daily weight gain significantly, and enhanced calcium and phosphorus digestibility significantly. Villi height to crypt depth ratio increased in the duodenum and jejunum at day 42 and this ratio increased in ileum at day 21 (Gao *et al.,* 2008). In another study, the results showed improvement in body weight and feed conversion in birds that fed *Saccharomyces cerevisiae* cell wall compared to the control group. These enhancements in body weight and feed conversion are due to the effects of yeast cell wall on intestine morphology which determined by villi height, especially the first 7 days of the bird's life (Santin *et al.,* 2001). An increase in body weight and a decrease in feed conversion ratio, is due to potential effective compounds like glycine, fructose, inositol, galactose, and sucrose which are produced by *Saccharomyces cerevisiae,* which are involved in metabolic pathways, including glycine, serine, and threonine metabolism (Sun *et al.,* 2019). In other studies, adding live yeast improved the body weight gain of the chickens and the feed conversion ratio significantly compared with control group (Tabidi *et al.,* 2013; and Eltazi *et al.,* 2014). *Saccharomyces cerevisiae* enhanced body weight gain in chicken groups that fed 0.1%, 0.2%, and 0.3% *Saccharomyces cerevisiae,* as the weight gain values were 1830g, 1902g, and 2011g respectively compared to 1636g in the control group (Tabidi *et al.,* 2013). Feed conversion ratio increased as well, as the ratios were 2.1, 1.9, and 1.8 respectively in birds' groups fed with 0.1%, 0.2%, and 0.3%

Saccharomyces cerevisiae compared to 2.3 in the control group and the differences were significant, while there was no significant effect of adding the yeast to broiler diet on feed intake (Tabidi *et al.,* 2013). Adding *Saccharomyces cerevisiae* to broiler chicken's diets at certain levels could lower mortality ratio significantly compared to mortality ratio in the control group (Eltazi *et al.,* 2014). Scientists explain the low mortality in yeast fed is attributed to the enhancement of immune system and disease infections modulated by competing pathogens or supporting the beneficial microbiota (Devegowda *et al.,* 1997; Line *et al.,* 1997; Spring *et al.,* 2000; Stanley *et al.,* 2004).

Prebiotic

Prebiotics were introduced for the first time in 1995 by Gibson and Roberfroid as a Nonee-digestible food ingredient that improves host health beneficially by stimulating the growth and/or activity of one or a limited number of selected beneficial bacteria in the colon. There are specific criteria of the product to be considered as prebiotic, such as: the product must resist stomach pH, should not be hydrolyzed by the enzymes of the host, should be available for the intestinal microbiota and could be fermented by them, can selectively stimulate the intestinal microbiota growth and/or stimulate their activity to improve the health of the host (Gibson *et al.,* 2010).). Multiple kinds of oligosaccharides and Nonee-starch oligosaccharide are counted as prebiotics such us; fructooligosaccharide, Mannanoligosaccharide, galactooligosaccharide, maltooligosaccharide, xylooligosaccharide, glucooligosaccaride, soya-oligosaccharide, isomaltooligosaccharide, lactulose, lactitol, inulin and pyrodextrins (Patterson and Burkholder, 2003; Steiner, 2006).

Yeast Cell Wall (MannanOligoSaccharide (MOS) and ß-1, 3-glucan)

Two layers form the YCW: the outer layer which is mainly composed of manno-proteins, and an inner layer which is approximately 50-60% of the cell wall dry weight. The inner layer of YCW consists of ß-1, 3-glucan. The inner layer provides the mechanical support (Klis *et al.,* 2002). The cell wall matrix of *Saccharomyces cerevisiae* consists of 40% manno-proteins of the dry mass. Mannan alone, forms 31% of the dry mass of the yeast (Klis *et al.,* 2002). In addition to Maannan-oligosaccharide (MOS) and glucan, YCW consist of ash, lipid, and protein (Northcote and Horne, 1952). Producing YCW occurs by removing the cell wall of the yeast completely, which results in a final product that has higher digestibility in addition of higher protein content (Tukmechi and Bandboni, 2014), and it is the way that Phileo Safmannan® is derived. It is difficult to purify the YCW more than 65% per fraction, which majority contain glucan, mannan, and protein (Kwaitkowski and Kwaitkowski, 2012). Feeding MOS to broiler chickens showed significant improvement at P= 0.02 in body weight, FCR, and mortality, which the enhancement percentages were 1.61%, 1.99%, and -21.4 respectively compared to the control group (Hooge, 2004). Feeding β-glucan (60 ppm) to broiler chickens could improve performance and be counted as an antibiotic alternative in poultry production (Moon *et al.,* 2016). Adding β-glucans to chicken's diet could stimulate specific and Nonee-specific immune responses in addition of chicken growth improvement (Vetvicka and Vetvickova, 2014; Rajapakse *et al.,* 2010). Schwartz and Vetvicka (2021) suggested that optimal β-glucan mixtures could be added to poultry feed to obtain ideal growth performance, get the desired anti-inflammatory and immunomodulatory activity, and

promote intestinal morphology and histology health. Adding YCW to broiler chicken's diets showed enhancement in feed efficiency versus *Echerichia coli.* The enhancement in feed efficiency attributed to an increase in immune response to microbial challenge (Morales-lopez and Brufau, 2013). The manno-protein particles in YCW are responsible to improve the immunity of the animals, which supplied with the YCW additive products (Ha *et al.,* 2006).

Adding yeast protein concentrate to broiler chickens' diets improved body weight, feed conversion ratio and immune response at day 35 significantly in broiler chickens that were exposed to *Salmonella Enteritis* (Haldar *et al.,* 2011). Feeding yeast protein concentrate to heat stressed broiler chickens could modulate circulatory levels of thyroid and cortisol (increase T3 and decrease cortisol significantly) which enhance the production performance (Haldar *et al.,* 2011). Adding YCW to broiler chicken's diets improved feed conversion from 1.74 in the control group to 1.70 in the treatment group significantly at $P \leq 0.01$, and improved villi height in the intestine ($P = 0.07$), resulting in improved broiler performance (Pascual *et al.,* 2020). Using MOS as feed additive to broiler chicken's diets improves intestinal health and immunity, in addition to improving the productivity versus *Clostridium perfrengins* (Caly *et al.,* 2015; and Fowler *et al.,* 2015). Feeding YCW to poultry could improve gut health and enhance the immune system activities of poultry as well (Świątkiewicz *et al.*, 2014). Fowler *et al.,* (2015) stated that the optimal dose of supplied YCW is 250 ppm. This supplement enhanced the growth rate 15% and enhanced feed conversion 10%. The productivity performance of the broiler chickens enhanced by improving the body weight and FCR. Therefore, YCW

additive is a promising alternative to antibiotics. In another study, adding 0.5% of YCW to broiler chicken's diets while exposed to heat stress for 42 days enhanced the chicken's performance (higher weight gain, lower feed conversion, and less mortality) compared to the control group without additive (Sohail *et al.,* 2012). The effect of YCW on the performance of broiler chickens and gut health was compared to *Clostridium perfringens.* The results showed the ileal *Clostridium perfringens* count in YCW group was lower than the other groups (Abudabos and Yehia, 2013). In addition, feeding whole yeast or YCW could enhances the villi height, and the ratio between villi height and crypt depth and correspondingly improves growth and performance occurs. Zhang *et al.*, (2005) proved in a study that ileal villi height and VH/CD ratio were higher in the additive groups (whole yeast and YCW) compared to yeast extract and control groups. In both whole yeast and YCW groups, improvement in growth performance of birds occurred. Safmannan® from Phileo®, is an example for prebiotics derived from YCW. XPC® and XPC-Ultra® concentrate from Diamond V® are examples of postbiotic additives to broiler chickens, as it contains mannan and glucan from the YCW.

Postbiotic

Postbiotic is a word derived from the Greek for 'post', meaning after, and 'bios', meaning life. It is defined by International Scientific association of Probiotics and Prebiotics (ISAPP) as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host". Effective postbiotics should contain inactivated microbial cells or cell components, which might or might not contains metabolites. Postbiotics are characterized by improving the health of the

animal, and acts as a potential mechanism to improve health. They should be safe and may be developed by different microorganisms (Salminen *et al.,* 2021). According to this definition, postbiotics could be made of diverse microorganisms to be applied in different body sites in different animals, plant or human, to encourage health improvement in different scientific areas associated with animals and plants. The term postbiotic is correctly referred to as a substance that derived after the microorganisms' death or inactivation (Vinderola *et al.,* 2022). Postbiotic products may be whole dead cells or fragments of microbes, such as cell walls. In addition, postbiotic products could contain microbe-produced substances like metabolites, proteins, or peptides, which are associated with overall health effect (Vinderola *et al.,* 2022). Postbiotics are one of the products that could be used to improve the intestinal microbiota and have increased in research recently. Postbiotics could affect health positively directly or indirectly by the beneficial substances that are released from microorganisms through metabolic activity. Postbiotic products risks are low because there are no living microorganisms involved (Zolkiewics *et al.,* 2020).

Adding postbiotic products to broiler chicken's diets could enhance the performance of heat stressed chickens. The results from one research showed significant improvement in broiler performance. Total body weight in one of postbiotic groups (RI11) were significantly higher compared to negative control, positive control (oxytetracyclin), and ascorbic acid groups. The total body weights were; 2951.75, 2795.11, 2717.52, and 2735.52 respectively. Likewise, daily gain weight was significant higher in postbiotic group compared to the negative control, positive control, and

ascorbic acid groups. The feed conversion ratio was significantly lower (1.61) in the same postbiotic group compared to the negative control, positive control, and ascorbic acid groups which they were 1.70, 1.72, and 1.72 respectively (Humam *et al.*, 2019). Adding postbiotic to broiler chicken's diets could improve villi height and decreased crypt depth significantly (Danladi *et al.,* 2022). Villi height in duodenum, jejunum and ileum improved significantly in postbiotics groups compared to the negative control, positive control, and antibiotic groups. Significant decrease in *Salmonella* count and significant increase in *lactoBacillus* count was documented in cecum in postbiotic groups compared to the negative and positive control groups (Humam *et al.,* 2019).

Clostridium perfringens

Clostridium perfringens is a rod-shaped spore forming bacteria that is encapsulated and Nonee-motile. It is an anaerobic Gram-positive which causes enteric disorder in animals and humans (Songer, 1996; Khelfa *et al.,* 2012). *Clostridium perfringens* produces multiple toxins and enzymes that affect health and production of poultry (Immerseel *et al.*, 2004). It can be found in the feed, feces, eggshell fragment, poultry litter, soil, dust and intestine tract of poultry (Craven *et al.*, 2001). *Clostridium perfringens* inhibits chicken's gut naturally, but the disease does not happen unless inducing factors are present, and colonization of *Clostridium perfringens* occurs early in the life of poultry (Craven *et al.,* 2000; and Craven *et al.*, 2001). According to the toxins production types, *Clostridium perfringens* strains are classified into classes A, B, C, D and E; these toxin types produce toxins α (alpha), ß (beta), ε (epsilon), and I (iota), respectively (Songer, 1996; Petit *et al.,* 1999). Type A *Clostridium* strains produce alpha

toxins while Type C clostridium strains produce both alpha and beta toxins as Petit et *al.*, (1999) mentioned, and they are both affect poultry production and responsible for lesions and symptoms. As a fact in poultry production, *Clostridium perfringens* is the most significant cause of necrotic enteritis (Songer, 1996). The high rate of adhesion by *Clostridium perfringens* to the intestinal mucosa led to necrotic enteritis and ulcerative enteritis (Williams, 2005).

Infection with *Clostridium perfringens* could cause clinical necrotic enteritis or subclinical necrotic enteritis. The clinical necrotic enteritis is characterized by diarrhea, anorexia, ruffled feathers, depression and sudden death (Freedman *et al.,* 2015). The subclinical type of necrotic enteritis is characterized by reduction in production, shallow diarrhea, and low mortality (Freedman *et al.*, 2015; Van Immerseel *et al.*, 2009).

Necrotic enteritis caused by *Clostridium perfringens* has a high cost in poultry production economically. Necrotic enteritis causes higher economic losses recently, especially after banning the usage of antimicrobial growth promoters in poultry feed by many countries (Abd El-Hack *et al.*, 2021). Inhibiting of poultry intestine by *Clostridium perfringens* can cause necrotic enteritis with presence of one or more influencing factors, such as: physical damage of intestine can occur by eating litter or fibrous material in the diet that can modify the mucosal lining (Williams, 2005). Presence of *Coccidia* or *Eimeria* can cause intestinal damage (Petit *et al.,* 1999: Immerseel *et al.,* 2004) and that can lead to necrotic enteritis combined with the presence of *Clostridium perfringens.* Necrotic enteritis could be induced by specific feed forms more than others. Feeding pellets to the birds instead of mash diet led to higher feed digestibility and a lower

Clostridium perfringens number in the intestinal tract (Engberg *et al.,* 2002). Mortality in birds fed with a fine ground diet were higher compared to birds fed with a coarse diet (Branton *et al.,* 1987). Diet composition could affect directly the onset of necrotic enteritis in broiler chickens. For instance, wheat diet rich diet in comparison to a corn diet, has high indigestible components and water-soluble Nonee-starch polysaccharides. The same with rye barley, diets which contain high indigestible components (Riddell and Kong, 1992) that affect the wellbeing of intestines showed feeding broiler chickens a contaminated corn-based diet with *Clostridium perfringens* compared to broiler chickens that were fed a high concentration of wheat, rye or barley diet resulted in higher mortality in the wheat, rye or barley animals' groups compared to the corn diet group (Riddell and Kong, 1992). Immunosuppression could increase *Clostridium perfringens* outbreaks in birds, which happens due to exposure to primary infection such as chicken anemia virus, infectious bursal disease, or Marek's disease. (Williams, 2005).

Salmonella Typhimurium

Salmonella is a Nonee-spore forming, gram-negative *Bacillus*, motile, aerobic to facultative anaerobic bacterium (Underwood *et al.,* 2015). It is an enteric bacterium which causes foodborne disease affect humans. It is a typical zoonotic disease which occurs in poultrys and causes losses due to mortality, inhibit growth rate and reduce egg production (Rebollada-Merino *et al.,* 2020; Dar *et al.*, 2017; Anderson and Kendal, 2017).

Vaccination with live, weaken strains of *Salmonella* could be used in poultry, but they have inconstant efficacy (Acevedo-Villanueva *et al.,* 2021; Berghaus *et al.*, 2011,

Dorea *et al.,* 2010). Vaccination could protect against early *Salmonella* colonizing, but the protection against *Salmonella* will decrease gradually and need booster administration (Dorea *et al.,* 2010; Jia *et al.,* 2020). Since feed additives are used for their health promoting effects through enhancing immunity, protection against pathogens by several mechanisms, they could be used to reduce *Salmonella* colonizing in poultry and enhance productivity (Tellez *et al.,* 2012; Hossain *et al.,* 2017). Chaney *et al.,* (2022) mentioned that adding postbiotics to the diets could reduce *Salmonella enterica* prevalence in broiler chickens houses as they found the significant reduction ($p<0.05$) in *Salmonella* prevalence in treatment houses for the three rearing cycles at 1.3%, 12.0%, and 2.4% compared to 7.3%, 22.0%, and 10.7% in control houses respectively. Prebiotics also could be used to reduce colonization of *Salmonella*. They could bind to the binding sites for pathogens to be excreted out of the intestine or by increasing short chain fatty acids concentrations which are not preferable by pathogens (Donalson *et al.,* 2008; Durant *et al.,* 2000). In addition to postbiotics and prebiotics, probiotics could be a promising treatment for Salmonellosis in broiler. Wolfenden *et al.,* (2007), found that gavaging *LactoBacillus*-based probiotic to broiler chickens at 4 x 10 cfu/mL could reduce cecal tonsil *Salmonella enteritis's* recovery compared to control group. Abd El-Ghani *et al.,* (2012), found that administrating probiotic to chickens could protect against *Salmonella* as vaccination does.

The specific objectives for this research were:

1. Evaluation of the effect of Phileo® Safmannan® prebiotic and Diamond V® yeast culture postbiotic (XPC® and XPC Ultra®) on the performance of broiler

chickens when challenged with *Clostridium perfringens* or exposed to *Salmonella Typhymurium*.

2. Evaluation the effect of Microsaf®, Envera Goplus® probiotics, Safmannan® prebiotic on starter broiler chicken's performance in birds subjected to bursa vaccine and *Clostridium perfringens* challenge.
3. Evaluation the effect of Safmannan ® prebiotic, Actisaf®, Envera Goplus® probiotics, and XPC® postbiotic on full term broiler chicken performance.
4. Evaluation of the mixture of different levels of Safmannan® prebiotic and *Bacillus* probiotic on birds subjected to bursa vaccine and *Clostridium perfringens* challenge.

CHAPTER II

EVALUATION OF THE EFFECT OF PHILEO® SAFMANNAN® PREBIOTIC AND DIAMOND V® YEAST CULTURE POSTBIOTIC (XPC® AND XPC ULTRA®) ON THE PERFORMANCE OF BROILER CHICKENS WHEN EXPOSED TO SAMONELLA TYPHYMURIUM

INTRODUCTION

Multiple types of feed additives have been used in poultry industry to improve production and prevent diseases. Feed additives must have the ability to prevent disease, improve the efficiency of growth, and enhance feed utilization to enhance performance of the poultry production (Pirgosliev *et al.*, 2019). Prebiotics and postbiotics are two types of feed additives that could be used in broiler production as growth promoters or antibiotic alternatives.

Prebiotics which was introduced by Gibson and Roberfroid (1995), as it is a Nonee-digestible food ingredient that improves host health by stimulating the growth and/or activity of one or a limited number of selected beneficial bacteria in the intestine. There are specific criteria of the product to be ideal prebiotics such as resistance to stomach pH, resistance to hydrolysis by the enzymes of the host, should be available for the intestinal microbiota, could be fermented by them, and can selectively stimulates the intestinal microbiota growth and/or stimulated their activity to improve the health of the host (Gibson *et al.,* 2010).). Multiple kinds of oligosaccharides and Nonee-starch polysaccharide are counted as prebiotics such us; fructooligosaccharide,

Mannanoligosaccharide, galactooligosaccharide, maltooligosaccharide, xylooligosaccharide, glucooligosaccaride, soya-oligosaccharide, isomaltooligosaccharide, lactulose, lactitol, inulin and pyrodextrins (Patterson and Burkholder, 2003; Steiner, 2006). According to Northcote and Horne (1952) the yeast cell wall of *Saccharomyces cerevisiae* (YCW) contains two major prebiotic polysaccharides which are; mannan (31%) and glucan (29%). Yeast cell wall components are involved in the modulation of the innate immune system by acting as pathogen associated molecular patterns (PAMPs) (Shashidhara and Devegowda ,2003). Feeding yeast cell prebiotic wall could enhance the gut health by enhancing mucus production and providing favorable conditions for beneficial intestinal bacteria like *lactoBacillus spp.*, *Bifidobacterium*, also competitive binding sites for pathogenic bacteria (Spring *et al.,* 2000; and Haldar *et al.,* 2011). Adding yeast cell wall to broiler chicken's diets showed enhancement in feed efficiency versus *Echerichia coli.* The enhancement in feed efficiency attributed to enhancement in immune response to microbial challenge (Morales-lopez and Brufau, 2013). Feeding yeast cell wall to broiler could improve the performance by improving feed conversion ratio (Pascual *et al.,* 2020). Yeast cell wall contains manno-proteins and ß-1, 3-glucan prebiotics (Klis *et al.,* 2002) which both contribute to intestinal morphology and poultry production improvement. The manno-protein in yeast cell wall could improve the immune response in animals (Ha *et al.,* 2006). Feeding Mannan-oligosaccharide prebiotic, which is a part of yeast cell wall, to broiler chickens enhanced body weight, feed conversion ratio, and mortality significantly compared to the control group (Hooge, 2004). Adding yeast

protein to broiler diet could enhance productivity through significant improvement of body weight and feed conversion ratio, in addition of significant enhancement of immune response of challenged broilers with Salmonella Enteridis (Haldar *et al.*, 2011). Postbiotics are a product which contains inanimate microorganisms and/or their components that leads to health improvement of the animal health. Postbiotics should have a potential mechanism of action to improve health and must be safe and could be created by different microorganisms (Salminen *et al.,* 2021). Ideally, postbiotics should be inactivated microbial cells or cell components. In addition, they may contain the end products of the metabolism activities of the microorganisms (metabolites) which lead to significant health benefits (Salminen *et al.*, 2021). Postbiotic products have been used in poultry industry as feed additives to enhance production as they reflect positive improvement on growth parameters and immune response enhancement. Significantly higher final body weight, total weight gain, average daily gain, and feed conversion ratio in broiler chickens that fed postbiotic than the birds in the other groups, as Humam *et al.*, (2019) proved in their study. Humam *et al.,* (2019) added postbiotic during finisher period to treatment groups. Adding postbiotics to broiler chicken's diets could improve villi height and decreased crypt depth significantly (Danladi et al., 2022), which could be a factor in improving the health of the chickens and promotes productivity. Intestinal morphology could be affected by microflora, thus villi height and crypt depth which both are indicators for intestinal health and functionality (Forder *et al.,* 2007; and Wang and Peng, 2008). Dahyia and Nagim (2022) mentioned that maintaining healthy balance

of microbiota is important to preserve normal physiology, metabolism, and immunity to prevent disease.

Adding postbiotics to broiler chicken's diets showed reduction in *Salmonella Enterica* incidence in broiler chicken's houses (Chaney *et al.,* 2022). The mechanism of prebiotics (a component of yeast cell wall) to reduce *Salmonella* colonization occur through binding to the binding sites for pathogens to be excreted out of the intestine, or by increasing short chain fatty acids concentrations which are not preferable by pathogens (Donalson *et al.,* 2008; Durant *et al.,* 2000). Therefore, this study is aiming to: 1) use yeast cell wall prebiotic of *Saccharomyces cerevisiae* and yeast postbiotic in different levels to study their effects on broiler performance during challenging with *Salmonella Typhimurium* in the first 10 days of chickens' life, 2) studying the effect of adding yeast cell wall prebiotic and yeast postbiotic to broiler diet on *Salmonella Typhimurium* count in ceca.

MATERIAL AND METHODS

Birds were housed at the Southern Plains Agricultural Research Center, United States Departments of Agriculture and the study was approved by the Texas A&M institutional Animal Care and Use Committee (IACUC 2014 - 0030) and the Animal Care and Use Committee at the Southern Plains Agriculture Research Center**.**

Experimental Design and General Procedure

A total 240 Ross 308 (origin: Sanderson Farms) newly hatched broiler chicks were distributed among 2 stainless steel battery brooder units (48 pens; 5 birds per pen) at United States Department of Agriculture (USDA) in College Station, Texas. A total of

6 treatments with 8 replicates for each treatment were randomly assigned to 48 individual pens for housing. The study continued for 10 days. All groups were fed on an industry type corn-soy starter diet. *Saccharomyces cerevisiae* cell wall and yeast postbiotic product in different concentrations were fed to the chickens in four treatment groups; XPC- Ultra® 625 ppm, XPC® 1250 ppm, Safmannan® 125 ppm, and Safmannan® 250 ppm. The last two groups fed the basal diet, but one of them was exposed *Salmonella Typhimurium* while the other was not.

A commercial mash corn-soybean basal diet for starter broilers was prepared (Table 3.1) and then divided into six equal portions to create six treatments as mentioned before. The birds and the feed were weighted at day one and day 10 of the study. The inoculation *Salmonella Typhimurium* was done at day 3 by oral gavage for each bird using 0.5 ml of *Salmonella Typhimurium* broth dilution $1x10^7$ to all exposed treatments.

Table 2.1 Antibiotic alternative treatments and *Salmonella Typhimurium* exposure used in this experiment

Treatment Products[1]	Concentration ppm	Exposure[2]	Antibiotic Alternative
XPC-Ultra®	625	+	Postbiotic
XPC®	1250	+	Postbiotic
Safmannan®	125	+	Prebiotic
Safmannan®	250	+	Prebiotic
Exposed control	-	+	Noneee
Nonee-Exposed control	-	-	Noneee

[1]Safmannan® is obtained from primary culture and the purification of selected Saccharomyces cerevisiae proprietary strain sold by Phileo®. XPC® is a yeast culture Saccharomyces cerevisiae yeast grown on a media of processed grain by-products, roughage products, cane molasses, malt and corn syrup sold by Diamond V®. XPC-Ultra® is a concentrated version of XPC®.
[2]*Salmonella Typhimurium*

The rearing room temperature was regulated by the computer controlled building thermostat. No concomitant drug therapy was used during the study. Birds were observed daily with regard to the general flock condition, room temperature, lighting, water, feed, and other unanticipated events for the house, and mortality for all pens. Feed and water was offered to birds *ad libitum*.

Table 2. 2 Ingredient and calculated composition of basal diet

Ingredient	Percentage
Corn	62.24
Dehulled Soybean Meal	31.71
DL-Methionine	0.27
Lysine HCL	0.18
L-Threonine 98%	0.03
Soybean oil	1.99
Limestone	1.31
Biofos[1] TM	1.55
Salts	0.41
Trace Mineral[2]	0.05
Vitamins[3]	0.25
Calculated Nutrient Content (%)	
Protein	22.00
ME (Kcal/Kg)	3050
Crude Fat	3.77
Crude Fiber	2.14
AV phosphate	0.45
Calcium	0.90
Methionine	0.60
Met+Cys	0.96
Lysine	1.30
Threonine	0.85
Arginine	1.45
Tryptophan	0.26

[1]Mono-calcium phosphate

[2]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

[3]Vitimin premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

Samples Collection and Preparation

On the 10th day, the remaining feed from each pen was weighted to find the feed consumption and feed conversion for all six treatments. All 5 birds from each pen were

weighted for performance calculations then they were killed by CO_2 gas as specified within IACUC permit, and all 5 birds per pen were taken for *Salmonella Typhimurium* plating numeration. Approximately 0.5 gm of ceca content from each bird was collected and diluted in Phosphate buffered Saline (PBS) for plating later the same day. Ceca samples were diluted using a 10x dilution series, and then placed onto Xylose-Lysine-Tergitol 4 (XLT-4) Agar treated with Novobiocin and Naladixic acid for use as a selective growth media. Enumeration counts were determined by visual identification of colonies after 24 hours of incubation.

Two birds were selected randomly to take the ceca swabs. The swabs were preserved in peptone water and sent to (Phileo®) for ROKA Bioscience analysis (Roka assays target ribosomal RNA (rRNA), to detect pathogens in the sample.

Bird weight and feed consumption were recorded in grams by pen at day 0 and 10 of the experimental period. Performance variables that were measured in this study include Body Weight (BW), weight gain per bird (WG), feed conversion ratio (FCR), mortality, productivity index (PI) with mortality, and PI without mortality. The PI was calculated using the following mathematical formula:

PI= (100-Mortality) × (Body Weight/1000)/Bird age/FCR×100.

The prebiotics used for this experiment was YCW derived from Saccharomyces cerevisiae, Safmannan® (Phileo-Lesaffre Animal Care, Milwaukee, WI, USA). Safmannan® prebiotic was used in two levels for two different treatments. Two yeast postbiotic products were used in this experiment as well; XPC® and XPC Ultra®, both

are from Diamond V®.

Statistical Procedures

One Way ANOVA test was used to analyze the data using the GLM procedure in SPSS^{TM} (IBM, Armonk, NY, USA). Means were separated using Duncan's multiple range tests at p-value ≤ 0.05 unless otherwise noted.

RESULTS AND DISCUSSION

Growth and Performance

The dietary treatments were formulated to make four treatment groups and two basal diets groups, five of the total six groups were challenged with *Salmonella* at day 3 and the experiment ended at day 10 when the final body weight, weight gain, feed conversion, feed to gain, mortality, and productivity index were calculated. The results, as shown in table (2.3), show there were no significant differences in final body weight between the treatments and both control groups. There were no significant differences between the weight gain in all treatments and positive control or negative control. These results were similar to what Morales-Lopez *et al.,* (2009) found, as they did not find significant differences in body weight between the treatment groups and control groups in the two experiments when they added YCW to the chicken's diet. The same results were documented in another study when no significant differences were documented in final body weight or weight gain between the postbiotic group and the control group (Danladi *et al.,* 2022). Pascual *et al.,* (2020), found no significant differences in total weight gain between Safmannan® group and the control group. The absence of

significant differences might be due to the short time of the present experiment, when the broiler chicks do not acquire many grams on their weight, and the weight gain will be faster in the later days of the animal's life.

No significant differences between the additives groups and the control groups in FCR. Danladi *et al.,* (2022) found similar results in their study, as they documented there were no significant differences between FCR of treatment groups and FCR of control group when they added postbiotic products to broiler chickens' diet, but there were significant differences in FCR between treatment groups themselves. Other studies showed the opposite results, as in a study was performed by (Pascual *et al.,* 2020), feed conversion showed significant enhancement in Safmannan® group compared to control group in period 3 (28-44 d), which the present study did not last that long. Feed conversion improvement was because of weight gain enhancement during this period (day 28-44), while no differences recorded in feed intake between YCW and control groups. That means enhancement in nutrient availability in yeast group compared to control group. Haldar *et al.,* documented that total weight gain was significantly improved in two yeast treatments compared to control group, while one yeast treatment improved weight gain but not significantly compared to control group (2011). All three yeast treatments showed significant improvement in FCR compared to control group (Haldar *et al.,* 2011).

In spite of the beneficial effect of antibiotic alternatives, their effects are not consistent and the results vary from farm to farm. (Kim & Lillehoj, 2019). Studies showed that the effects of postbiotics of YCW lack consistency, as some studies showed

contrary results to what mentioned earlier, significant enhancement in performance of broiler occurred when YCW or postbiotic products were added to broiler diet. Fowler *et al.,* (2015) documented significant enhancement in total body weight at day 21 in Safmannan® treatment compared to control group. They documented numerical enhancement in FCR of Safmannan treatment compared to control group, but the difference wasn't significant. The same results were documented in (Hashim *et al.,* 2019) studuy, they found significant enhancement in total body weight and weight gain in all YCW treatments compared to the control group. FCR did not show any differences among the treatment groups compared to the control group in their study.

Mortality percentage in this study did not show significant differences between all groups. The same results were documented in some studies (Fowler *et al.*, 2015, Hashim *et al.,* 2019, Danladi *et al.,* 2022).

Table 2.3 Day 10 Production Performance

Treatment	Final Body Weight (g)	Weight Gain (g)	Feed to Body Weight	Feed to Gain	Mortality %
Cultured Yeast Cell Wall Products (postbiotic)					
XPC-Ultra® 625	257± 19[a]	212± 19[a]	1.16± 0.03[ab]	0.95± 0.03[a]	0 ± 0
XPC® 1250	262± 14[a]	216± 13[a]	1. 15± 0.02[a]	0.95± 0.01[a]	0 ± 0
Yeast Cell Wall Products (prebiotic)					
Safmannan® 125	248± 30 [a]	203± 30 [a]	1.19± 0.04[b]	0.96± 0.04[a]	5 ± 14
Safmannan® 250	256± 15[a]	211± 15[a]	1.18± 0.05[ab]	0.97± 0.04[a]	0 ± 0
Control Groups (no antibiotic alternative)					
Exposed control [1]	267± 13[a]	222± 15[a]	1.16± 0.04[ab]	0.97± 0.03[a]	0 ± 0
Noneee-exposed control [2]	261± 9[a]	215± 8[a]	1.16± 0.02[ab]	0.96± 0.02[a]	0 ± 0

[a,b] Means ±Standard Deviation within a column with no common superscript differ based on Duncan's multiple range test. For this experiment there was no difference between the challenged and unchallenged birds nor was there a difference between the yeast culture postbiotics or yeast cell wall prebiotics. ANOVA and Duncan's mean separations are therefore shown based on a one-way ANOVA.
[1] Exposed to *Salmonella Typhimurium*.
[2] Not exposed to *Salmonella Typhimurium*.

As table 2.4 shows, PI of Safmannan® 125 was significantly lower than both positive and negative control groups and significantly lower than PI of XPC® group as well. But when PIs were calculated without mortality values (which were not significant different in all groups), PI did not show differences in all groups. Fowler et al., (2015) and Hashim *et al.,* (2019) did not find significant differences in PI between YCW treatments and control groups.

Table 2.4 Day 10 Productivity Index With & Without Mortality in Safmmanan125 Group

Treatment	PI With Mortality	PI Without Mortality
Cultured Yeast Cell Wall Products (postbiotic)		
XPC-Ultra® 625	223 ± 17 [ab]	223 ± 17 [a]
XPC® 1250	229 ± 15 [a]	229 ± 15 [a]
Yeast Cell Wall Products (prebiotic)		
Safmannan® 125	199 ± 42 [b]	210 ± 31 [a]
Safmannan® 250	217 ± 18 [ab]	217 ± 18 [a]
Control Groups (no antibiotic alternative)		
Exposed control [1]	231 ± 17 [a]	231 ± 17 [a]
Nonee- Exposed control [2]	225 ± 10 [a]	225 ± 10 [a]

[a,b] Means ±Standard Deviation within a column with no common superscript differ based on Duncan's multiple range test. For this experiment there was no difference between the challenged and unchallenged birds nor was there a difference between the yeast culture postbiotics or yeast cell wall prebiotics. ANOVA and Duncan's mean separations are therefore shown based on a one-way ANOVA.
PI (Productivity Index) = (VB*AW)/ (FC*AA)
VB= (Final number of birds/Initial number of birds)*100
AW= Birds average weight
FC=Total consumption of feed/ total weight gain
AA=Age of Animal
[1] Exposed to *Salmonella Typhimurium*.
[2] Not exposed to *Salmonella Typhimurium*.

Salmonella Typhimurium Count

Counting *Salmonella Typhimurium* in plates showed significant decrease in Safmannan® 125 ppm group compared to all other challenged groups with *Salmonella Typhimurium* as table (2.5) shows. Haldar *et al.*, (2011) found that one of yeast treatments significantly lowered *Salmonella Typhimurium* count in the plates compared to the control group and the other yeast product treatments groups.

No significant differences were recorded in ROKA count between all exposed groups to *Salmonella Typhimurium*, and that might because ROKA is a molecular method which detect ribosomal RNA of Salmonella in general (Hu *et al.*, 2018), which means it detect live and dead *Salmonella Typhimurium* and other *Salmonella* species in the samples.

Table 2.5 Day 10 *Salmonella Typhimurium* Numeration by Plating & ROKA[1]

Treatment	Count in plates (CFU log10)/ gm	ROKA[1] count (CFU log 10)/ml
Cultured Yeast Cell Wall Products (postbiotic)		
XPC-Ultra® 625	6.01 ± 1.16 [c]	4.93 ± 0.60 [b]
XPC® 1250	5.67 ± 1.13 [c]	4.77 ± 0.55 [b]
Yeast Cell Wall Products (prebiotic)		
Safmannan® 125	5.07 ± 1.83 [b]	4.87 ± 0.57 [b]
Safmannan® 250	6.04 ± 1.05 [c]	5. 02 ± 0.22 [b]
Control Groups (no antibiotic alternative)		
Exposed control [2]	5.77 ± 1.39 [c]	5.01 ± 0.31 [b]
Nonee- Exposed control [3]	0.17 ± 1.10 [a]	3.75 ± 2.13 [a]

[1]Roka assay target ribosomal RNA (rRNA) to detect pathogen in the sample.
[a,b,c] Means ±Standard Deviation within a column with no common superscript differ based on Duncan's multiple range test. For this experiment there was no difference between the challenged and unchallenged birds nor was there a difference between the yeast culture postbiotics or yeast cell wall prebiotics. ANOVA and Duncan's mean separations are therefore shown based on a one-way ANOVA.
[2] Exposed to *Salmonella Typhimurium*.
[3] Not exposed to *Salmonella Typhimurium*.

CONCLUSION

In conclusion, adding yeast cell wall prebiotic and yeast postbiotic products to the starter diet of broiler chickens did not enhance production performance at day 10 of the chicken's life, as there were no significant differences between all groups in total body weight, daily gaining weight, FCR, or mortality ratio during the first 10 days of the bird's life while exposing to *Salmonella Typhimurium*. Safmannan® 125 group showed significant decrease in *Salmonella Typhimurium* count on the plates compared to XPC® 1250 group, which means it is more effective in controlling the disease and could work as antibiotic alternative.

CHAPTER III

EVALUATION OF THE EFFECT OF PHILEO® SAFMANNAN® AND DIAMOND V® YEAST CULTURE ON THE PERFORMNACE OF BROILER CHICKENS WHEN CHALLENGED WITH *CLOSTRIDIUM PERFRINGENS*

INTRODUCTION

Clostridium perfringens is an anaerobic, Gram-positive, rod-shaped, spore forming bacteria that is encapsulated and Nonee-motile and causes enteric disorder in animals and humans (Songer, 1996; Khelfa *et al.,* 2012). *Clostridium perfringens* can be found in the soil, dust, feed, feces, eggshell fragment, poultry litter, intestine tract of poultry (Craven *et al.*, 2001). *Clostridium perfringens* inhibits chicken's gut naturally, but because it produces multiple toxins and enzymes it could affect poultry production (Immerseel *et al.,* 2004). The disease does not happen without contribution of inducing factors. Colonization of *Clostridium perfringens* occurs early in the life of poultry (Craven *et al.,* 2000; Craven *et al.,* 2001). Necrotic enteritis and ulcerative enteritis occur due to *Clostridium perfringens* adhesion to the intestinal mucosa, if the adhesion occurred extensively (Williams, 2005). Necrotic enteritis causes high economic losses in poultry production, especially after the recent banning of antimicrobial usage as growth promoters in poultry farms (Abd El-Hack *et al.,* 2021). Adding antibiotic alternative is an option to elevate the stress occurs because of the pathogens infection, and *Saccharomyces cerevisiae* products are options for this purpose, in addition to their growth promoting effect as well according to the researchers (Ahiwe *et al.,* 2021). Yeast

prebiotics and postbiotic products could be used as antibiotic alternatives as the scientist's defined prebiotics as a Nonee-digestible food ingredient that improves host health by stimulating the growth and/or activity of selected beneficial bacteria in the intestine (Gibson and Roberfroid, 1995), and yeast cell wall contain two kinds of prebiotic oligosaccharides which are glucan and mannan. While postbiotics were defined as a product contains inanimate microorganisms and/or their components that leads to health improvement of the animal. Postbiotics improve health through certain potential mechanism of action and are characterized to be safe and could be created by different microorganisms (Salminen *et al.*, 2021).

Feeding broiler chickens on a diet that contains yeast culture led to significant enhancement in weight gain and improved calcium and phosphorus digestibility significantly (Gao *et al.,* 2008). Hashim *et al.,* documented that the effects of adding yeast cell wall (YCW) to broiler diet vary while challenging with *Clostridium perfringens* depending on the type of additive (2017). They found adding yeast cell Mannan-oligosaccharide (MOS) and purified YCW led to body weight enhancement in both groups compared to challenge control group, while adding semi purified YCW or purified beta-glucan caused body weight decrease compared to challenged control group at day 21 of birds' age. In six studies performed by Fowler *et al.,* (2015) proved 15% enhancement in growth and a 10% reduction in FCR when a blend of two YCW products added to broiler diet. They suggested that YCW products could be used as growth promoters in broiler farms and the optimum dose of Safmannan® is 250 ppm. Johnson *et al.*, (2020) found that adding YCW products to challenged broiler chicken's

diets with necrotic enteritis enhanced weight gain compared to challenge group at day 21. Feeding MOS and β-glucan prebiotics (which are part of yeast cell wall) are proved to enhance broiler performance (Hooge, 2004; and Moon *et al.*, 2016). Enhancement of broiler chicken production could occur through enhancement of immune response during supplying YCW to the animal, thus, improvement of animal health occurs (Ha *et al.*, 2006; and Świątkiewicz *et al.*, 2014).

Postbiotics produced from *Saccharomyces cerevisiae* could be used in broiler chicken's diet as an antibiotic alternative, as they contain YCW and some metabolites produced by the yeast which all contribute in animal health improvement (Sun *et al.*, 2019). Postbiotic products could be used safely in broiler chicken production as Zolkiewics *et al.*, (2020) described, postbiotic products could affect health directly or indirectly by the beneficial substances that are released from microorganisms. Because there are no living microorganisms involved, the risks of postbiotic are low (Zolkiewics *et al.*, 2020). Sometimes, yeast additives shows different effects on broiler, as Johnson *et al.*, (2020) showed that semi-purified YCW, beta glucan, or manno-protein groups did not show significant differences in weight gain at day 21 compared to the control groups when the animals were challenged with necrotic enteritis, while the combination group of beta glucan and manno-protien showed significant enhancement in weight gain at day 21 compared to challenged control group. Morales-López *at al.*, (2009) found no significant differences in body weight between yeast cell wall group and control group.

In this study, Safmannan® prebiotic from Phileo® by Lesaffre and XPC®, and XPC-Ultra® postbiotics from Diamond V® concentrate were added to broiler chicken's

diets while challenging with *Clostridium perfringens* to evaluate the pathogen negative effects on broiler chickens performance.

MATERIAL AND METHODS

The study was approved by the Texas A&M institutional Animal Care and Use Committee (IACUC 2014 - 0030) and the Animal Care and Use Committee at the Southern Plains Agriculture Research Center**.** Birds were housed at the Southern Plains Agricultural Research Center, United States Departments of Agriculture.

Experimental Design and General Procedure

A total 200 Ross 308 broiler chicks were randomly distributed between two stainless steel battery units (48 pens; 4 birds per pen). A total of 8 replicates for each group were sequentially assigned to pens such that each treatment was represented at least once for any given level of pens (4 levels). The standard Sanderson Farms Hatchery vaccinations were administered on the day of hatch.

Birds were vaccinated with a commercial Infectious Bursa Disease vaccine at day 10 of age. Challenge with *Clostridium perfringens* occurred on day 16 and 17 of the experiment. 3 ml oral gavage 10^7 CFU/ml per each challenged bird for all birds in challenged groups. The study was terminated on day 21. The specific treatments are shown below in table (3.1) as five groups were challenged with *Clostridium perfringens* while one group kept as Nonee-challenged negative group. XPC-Ultra® concentrated 625 ppm was added to the diet in first group, XPC® 1250 ppm added to the diet for the second group, Safmannan® 250 ppm was added to the diet for the third group,

Safmannan® 500 ppm was added to the diet for the fourth group, and the last two groups were fed basal diet without additives and kept as two control groups.

Table 3.1 Yeast prebiotic and postbiotics additives and *Clostridium perfringens* challenge used in the experiment

Treatment Products[1]	Concentration (ppm)	Challenge[2]	Antibiotic Alternative
XPC-Ultra®	625	+	Postbiotic
XPC®	1250	+	Postbiotic
Safmannan®	250	+	Prebiotic
Safmannan®	500	+	Prebiotic
Challenged Control group	0	+	Nonee
Noneee-Challenged Control group	0	-	Nonee

[1]Safmannan® is obtained from primary culture and the purification of selected Saccharomyces cerevisiae proprietary strain sold by Phileo®. XPC® is a yeast culture *Saccharomyces cerevisiae* yeast grown on a media of processed grain by-products, roughage products, cane molasses, malt and corn syrup sold by Diamond V®. XPC-Ultra® is a concentrated version of XPC®.

[2]Challenge with *Clostridium perfringens* occurred on day 16 and 17 of the experiment. 3 ml oral gavage 10^7 CFU/ml per each challenged bird for all birds in challenged groups.

Two stainless steel Battery Brooder Units were used in this study to raise the birds. Four birds were randomly allocated to each pen, (32 chicks for each treatment in eight replicates. Each pen was as a replicate (48 pens; 4 birds by pen, 2 sq ft per cage). The rearing room temperature was regulated by the computer controlled building thermostat. No concomitant drug therapy was used during the study. Diets were based on treatment addition to a corn-soy industry type basal

broiler starter diet (table 3.2). Birds were observed daily with regard to the general flock condition, room temperature, lighting, water, feed, and other unanticipated events for the house, and mortality for all pens.

Table 3.2 Ingredient and calculated composition of basal diet

Ingredient	**Percentage**
Corn	58.43
Soybean Meal	34.49
DL-Methionine	0.23
Lysine HCL	0.18
AV Blend 8500	2.76
Limestone	1.56
BioFos 16/21P	1.54
Salts	0.51
Vitamins Premix[2]	0.25
Trace minerals Premix[3]	0.05
Calculated Nutrient Content (%)	
Protein	22
ME (Kcal/Kg)	3050
Crude Fat	5.32
Crude Fiber	2.63
AV phosphate	0.45
Calcium	0.95
AV Methionine	0.53
AV Met+Cys	0.83
AV Lysine	0.19
Sodium	0.22
Potassium	0.86
Chloride	0.39

[1]Mono-calcium phosphate

[2]Vitamins premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

[3]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

Statistical Procedures

Data were analyzed as a complete randomized block design using the GLM procedure of SPSS. Blocks and treatments were used as fixed factors in the statistical model. Where significance was detected in the overall model for each dependent variable; means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

The results in three phases of the experiment showed different results according to the individual phase or accumulative calculation for the whole experiment.

Growth and Performance

Results listed in table (3.3) showed no significant differences between all groups at day 10 0f the experiment. No differences in body weight, weight gain, cumulative feed to body weight, phase feed to gain, PI, or phase mortality between yeast prebiotic and postbiotics additive groups, neither differences found between the additives groups and both control groups. These results were similar to what Morales-Lopez *et al.,* (2009) found. They did not find significant differences in body weight between the treatment groups and control groups in the two experiments when they added YCW to the chicken's diets. The same results were documented in another study when no significant differences were documented in final body weight, weight gain, nor FCR between the postbiotic group and control group (Danladi *et al.,* 2022). As well as Pascual *et al.,* (2020) found no significant differences in total weight gain or feed conversion index between Safmannan® group and control group at day 14. Whereas other studies proved

the opposite results, as Ahiwe *et al.*, (2019) showed that adding yeast cell wall product could enhance the body weight and feed conversion ratio at day 10 of chickens' age.

Table 3. 3 Day 10 Production Performance

Treatment	Body Weight (g)	Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	PI[1]	Phase Mortality %
Cultured Yeast Cell Wall (Postbiotic)						
XPC-Ultra® 625	262±21	223±21	1.00±0.02	1.18±0.04	247±26	0 ± 0
XPC® 1250	270±9	231±9	1.00±0.00	1.17±0.02	257±13	0 ± 0
Yeast Cell Wall Products (Prebiotics)						
Safmannan® 250	264±11	225±10	0.98±0.02	1.15±0.02	255±13	0 ± 0
Safmannan® 500	261±21	222±21	1.00±0.02	1.18±0.03	246±28	0 ± 0
Control Groups (No Antibiotic Alternatives)						
Challenged Chicks	256±15	217±15	0.98±0.01	1.16±0.02	246±16	0 ± 0
Noneee-Challenged Chicks	265±9	226±9	0.99±0.02	1.16±0.02	254±10	0 ± 0

± Standard Deviation

[1]PI = Productivity Index

At day 16 of the experiment, no significant differences in body weight, phase weight gain, FCR, or mortality percentage among the additive groups and the control groups as table (3.4) displays. The same results that Johnson *et al.,* (2020) found in their study, as they could not prove any significant differences at day 16 in body weight or weight gain between the groups. Additionally, there were no significant differences between FCR of YCW groups compared to challenge control group. In another study, adding YCW to broiler diet caused declining in both daily weight gain (P=0.04) and feed intake (P≤0.01) in the second phase of the experiment (14-28 d), but no effect on feed conversion was recorded (Pascual *et al.,* 2020). Hashim *et al.,* (2017) found that adding semi-purified YCW to broilers' diet decreased body weight and PI compared to control groups at day 16 of the study, while it did not affect FCR, or mortality percentage. In same study, the researchers found that adding purified YCW to broilers chicken's diets did not affect the performance of the chickens at day 16 of the experiment positively or negatively. The reason of not showing differences among the groups is, feed additives expected to enhance the production in stress situation to reach to the genetic potential growth of the animal, and this enhancement absence in performance occurred due to the stress factor was not exist before challenge which occurred at day 16 after performance parameters measurements were documented.

Table 3. 4 Day 16 Production Performance

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	Cumulative Feed to Gain	PI [1]	Phase Mortality	Cumulative Mortality %
Cultured Yeast Cell Wall (Postbiotic)								
XPC-Ultra® 625	566±45	282±25	1.139±0.015^{c}	1.27±0.02^{c}	1.23±0.02^{c}	295±29	0	0
XPC® 1250	566±18	296±9	1.133±0.012^{c}	1.25±0.02^{bc}	1.22±0.02^{bc}	310±13	0	0
Yeast Cell Wall Products (Prebiotics)								
Safmannan® 250	546±2	282±14	1.129±0.011^{bc}	1.27±0.02^{c}	1.22±0.01^{bc}	300±13	0	0
Safmannan® 500	545±42	284±21	1.136±0.011^{c}	1.26±0.02^{bc}	1.23±0.02^{c}	297±26	0	0
Control Groups (No Antibiotic Additives)								
Challenged Chicks	548±31	292±23	1.110±0.014^{a}	1.22±0.03^{a}	1.20±0.02^{ab}	306±21	0	0
Noneee-Challenged Chicks	548±17	283±14	1.117±0.021^{ab}	1.24±0.03^{ab}	1.20±0.02^{ab}	303±10	0	0

$^{a-c}$ Means within a row with no common superscript differ significantly (P<0.05)

± Standard Deviation

[1] PI= Productivity Index (PI= (100-MORT) × (BW/1000)/Bird age/FCR×100).

There were no significant differences in body weight at day 21 between all groups, as table (3.5) shows. No significant differences between all groups in phase weight to gain, phase feed to gain, cumulative feed to gain, cumulative feed to body weight, or productivity index. There was mortality in both Safmannan groups and in the negative control, but no significant differences in mortality ratio between the groups were recorded at day 21 of the experiment.

Morales-López *et al.,* (2009) did not record significant differences in body weight between groups when they added YCW. In another study, adding YCW tended to decrease body weight compared to control group at day 14 and 28 of age. The values of the decrease in body weight in group fed yeast were at day 14 was -2.6% at $P = 0.08$, and - 2.9% at $P = 0.03$ at day 28 (Pascual *et al.,* 2020). Johnson *et al.,* (2020) documented that adding YCW additive to broilers diet did not show significant differences in body weight at day 21 of the experiment compared to control group. In another study, adding semi-purified YCW while challenging with *Clostridium perfringens* did not enhance body weight, FCR, mortality percentage, nor PI compared to challenge group (Hashim *et al.,* 2017). In a study conducted by Hashim *et al.,* (2019), adding YCW products to broiler chicken's diets improved birds' weight and weight gain at day 21 of the chickens' age, but YCW additives did not affect feed to gain ratio, FCR, PI, nor mortality percentage compared to control group.

Table 3. 5 Day 21 Production Performance

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	Cumulative Feed to Gain	PI [1]	Phase Mortality %	Cumulative Mortality %
Cultured Yeast Cell Wall (Postbiotic)								
XPC-Ultra® 625	860±59	316±39	1.255±0.019[b]	1.48±0.11	1.317±0.02[b]	316±33	0	0
XPC® 1250	874±23	309±25	1.246±0.021[b]	1.46±0.08	1.304±0.02[b]	335±13	0	0
Yeast Cell Wall Products (Prebiotics)								
Safmannan® 250	858±25	312±20	1.240±0.020[b]	1.44±0.06	1.299±0.02[b]	320±28	3±9	3±9
Safmannan® 500	876±55	332±21	1.237±0.021[ab]	1.41±0.07	1.297±0.02[ab]	318±54	6±12	6±12
Control Groups (No Antibiotic Additives)								
Challenged Chicks	857±34	309±42	1.236±0.024[ab]	1.48±0.13	1.297±0.03[ab]	331±18	0	0
Nonee-Challenged Chicks	879±35	331±15	1.216±0.012[a]	1.38±0.01	1.273±0.01[a]	345±10	3±9	3±9

[a,b] Means within a row with no common superscript differ significantly (P<0.05).

± Standard Deviation.

[1] PI= Productivity Index.

CONCLUSION

There were no significant differences in performance parameters of broiler chickens at day 10, 16, or 21 of the experiment between YCW prebiotic or postbiotic additive compared to challenged and Noneee-challenged group. Some studies proved same results, like Johnson *et al.,* (2020) did not find significant differences at day 16 or 21 of the experiment in body weight or weight gain between the groups when YCW was added to the broilers' diet, and there were no significant differences between FCR of YCW group compared to challenged control group while challenging with *Clostridium perfringens*. Hashim *et al.,* (2017) showed no positive significant effect from adding Nonee-purified YCW to broiler diet on their performance.

As Kim and Lillehoj (2019) mentioned, antibiotic alternative lack consistency as in some Studies, adding yeast additives to broiler diets showed enhancement in production as (Fowler *et al.*, 2015; Hashim *et al.*, 2019).

CHAPTER IV

EVALUATION THE EFFECT OF MICROSAF®, ENVERA GOPLUS® PROBIOTICS, SAFMANNAN® PREBIOTIC ON STARTER BROILER PERFORMANCE IN BIRDS SUBJECTED TO BURSA VACCINE AND *CLOSTRIDIUM PERFRINGENS* CHALLENGE

INTRODUCTION

Using antibiotics in broiler chicken farms has had a significant effect in enhancing animal health and production by lowering occurrence of diseases and mortality. However, because of antibiotic-resistance which affect human health and animal production, public pressured and antibiotic banned from being used in animal farms as growth promoters. All of that led to actively search the antibiotic alternatives to improve poultry production. Some products like probiotics and prebiotics found to be used as antibiotic alternative feed additives to promote broiler production (Diarra and Malouin, 2014).

Probiotics are live microorganisms which improve animal health when administrated in adequate amount as feed additives. Animal health improvement occur through enhancing the microbial intestinal balance to get the desired benefits and improve animal health (FAW/WHO, 2002). Adding probiotics to poultry diets could enhance intestinal health through supporting the beneficial microbiota and suppressing harmful bacterial growth in the digestive tract (Jadhav *et al.*, 2015). Using probiotics in poultry diets can limit various infectious diseases, and the optimal effects of probiotics

can occur by appropriate selection of probiotic strains which are used as feed additives (Alagawany *et al.,* 2018).

Probiotic supplementation could support health and enhance production of poultry through multiple mechanisms, such as enhancement of intestinal microbiota, modulation of immune system, pathogen exclusion and prevention of colonization, alteration of ileal digestibility and total apparent digestibility coefficient, decrease in ammonia and urea excretion, improvement of growth performance (Jha *et al.,* 2020). The benefits of probiotics can occur directly in the gastrointestinal tract and indirectly through modulation of immune response in poultry (Krysiak *et al.,* 2021).

There are many microorganisms used to produce probiotics, such as the bacteria *Bifidobacterium spp.*, *Lactococcus spp*., *LactoBacillus spp*., and *Bacillus spp.* Yeast are used as well to produce probiotic products (Park *et al.,* 2016). In multiple studies, some *Bacillus spp.* showed enhancement in poultry health and production. Feeding broiler chickens on a diet which contains *Bacillus amyloliquefaciens* lead to significant improvement in growth performance and immunity, as an increase of body weight in addition to reduction of feed conversion ratio occurred (Ahmat *et al.,* 2021; Hong *et al.,* 2019; & Ahmed *et al.,* 2014). Improvement of growth performance of broiler chickens occur after feeding *Bacillus amyloliquefaciens* which could be an outcome of cecal microflora enhancement and intestinal morphology enhancement which allow better nutrient utilization (Lei *et al.,* 2015). *Bacillus amyloliquefaciens* as a feed additive led to feed conversion ratio (FCR) improvements compared to broiler chickens with both of *Eimeria maxima* and *Clostridium perfringens* significantly, as well as lowering mortality

rate compared to the challenged broiler chickens without feed additives. (Oliveira et al., 2019).

Bacillus pumilus probiotics could be used in poultry production earlier in life to enhance cecal microbiota, like; *Ruminococcaceae*, *LactoBacillus,* and *Bifidobacterium*, which can affect poultry performance due to the health promoting effects of these microorganisms (Bilal *et al.*, 2021). In addition of microbiota enhancement, *Bacillus pumilus* secrete antimicrobial substance (Hasan *et al.*, 2009). Those antimicrobial substances could have inhibitory effects against some pathogens (Chu *et al.,* 2019).

Bacillus licheniformis could be used as an antibiotic alternative to improve growth performance in poultry (Liu *et al.*, 2012). Adding *Bacillus licheniformis* to broiler diet could enhance daily weight gain and feed conversion ratio significantly compared to the control group (Liu *et al.,* 2012). *Bacillus licheniformis* could be considered as antibiotic alternative as it could be supplied to the broiler chickens to prevent necrotic enteritis caused by *Clostridium perfringens* (Knap *et al.*, 2010).

LactoBacillus probiotics showed enhancement effect on broiler chicken performance. In a study, adding *LactoBacillus* probiotic to broiler chickens could enhance FCR compared to control group at day 21. The values of FCR were 1.39 and 1.53 respectively in *LactoBacillus* and control groups (Chen *et al.,* 2017). In another study, adding *LactoBacillus reuteri* to broiler chicken diet improved FCR in weeks between 5th and 8th of the chickens age (Bhogoju *et al.,* 2021). *Lactobacilli* probiotic could improve body weight and weight gain when added to broiler diet (Fesseha *et al.*, 2021). *LactoBacillus sp.* could enhance intestinal villi permeability and improve

nutrients absorption which affect body weight positively (Pertiwi and Mahendra, 2021). *Lactobacilli* could be used as an antibiotic alternative because it has multiple mechanisms to inhibit pathogens like producing organic acids, producing hydrogen peroxide (H2O2), and producing bacteriocin (Taheri *et al.,* 2009).

Saccharomyces cerevisiae could be used as a probiotic and act as antibiotic alternative since it affects growth in broiler chicken positively. Yeast cell wall (YCW) products could be used as prebiotics as well to enhance broiler production (Ahiwe *et al.,* 2021). Adding yeast culture to broiler diet had significant positive effect on weight gain and significantly enhanced calcium and phosphorus digestibility, which ultimately supports growth (Gao *et al.,* 2008). The improvements that happens after feeding yeast products to broiler chickens could be attributed to the effects of yeast cell wall on intestine morphology (Santin *et al.,* 2001), as adding YCW to the animals' diet could increase the villi height in the intestine (Abudabos and Yehia, 2013), or the enhancement in performance could be referred to the effects of the metabolites that produced by *Saccharomyces cerevisiae* which are involved in metabolic pathways in the animal's body, including glycine, serine, and threonine metabolism (Sun *et al.,* 2019). Feeding YCW could have positive effect on broiler production since it consists of mannan-oligosaccharide (MOS) and β-glucan as these effects proved in studies. Adding MOS to broiler chicken's diets enhanced body weight, FCR, and mortality significantly in treatment group compared to control group (Hooge, 2004). β-glucan could improve chicken's performance and worked as an antibiotic alternative when added to poultry diet (Moon *et al.,* 2016). In addition of the production improvement, adding YCW to

broiler chicken's diets could improve gut health and enhance the immune response of poultry as well (Świątkiewicz *et al.*, 2014). Adding YCW prebiotic could work as antibiotic alternative which enhances production versus broiler chickens with *Clostridium perfringens* (Pascual *et al.*, 2020; and Fowler *et al.*, 2015).

In this study, Miscrosaf ® probiotic, Safmannan® prebiotic, from Phileo ® by Lesaffre, and Envera Goplus® probiotic (*LactoBacillus spp.*) are used to evaluate their effects on broiler performance versus broiler chickens with *Clostridium perfringens*. Microsaf ® contains *Bacillus amyloliquefaciens, Bacillus licheniformis,* and *Bacillus pumilus.*

MATERIAL AND METHODS

The study was approved by the Texas A&M institutional Animal Care and Use Committee (IACUC 2014 - 0030) and the Animal Care and Use Committee at the Southern Plains Agriculture Research Center**.** Birds were housed at the Southern Plains Agricultural Research Center, United States Departments of Agriculture.

Experimental Design and General Procedure

A total 288 Ross 308 broiler chicks were randomly distributed between two stainless steel battery units (48 pens; 6 birds per pen). A total of 8 replicates were sequentially assigned to pens such that each treatment was represented at least once for any given level of pens (4 levels). The standard Sanderson Farms Hatchery vaccinations were administered on the day of hatch.

At day 10, one bird from each pen was removed and the rest birds vaccinated with a commercial Infectious Bursa Disease vaccine to immune compromise the birds.

The challenge with *Clostridium perfringens* was done at days 16 and 17 (3 ml oral gavage 10^7 CFU/ml) after one bird was removed from each pen to get bird numbers down to 4 birds per pen as USDA approved AUP.

The chicks and feed were weighted on the first day, the 10^{th} day, day 16, and day 21 to evaluate the production performance. The study employed six treatments; five groups were challenged with *Clostridium perfringens* and the feed was supplemented with: Phileo Microsaf® 500g/M ton, Envera Goplus® 500g/ M ton, Envera Goplus® 500g/ M ton + Safmannan® 125 ppm, and Safmannan® 250 ppm. In addition of a challenged control consisted of the basal diet without yeast or probiotic product addition, and a Nonee-challenged control, which was fed the basal diet without *Clostridium* challenge. The specific treatments are shown in table (4.1).

Table 4.1 Probiotics and YCW treatments and *Clostridium perfringens* challenge used in the experiment

Treatment Products[1]	Concentration (ppm)	Challenge[2]	Antibiotic Alternative
Microsaf®	500	+	Probiotic
Envera Goplus®	500	+	Probiotic
Envera Goplus® + Safmannan®	500 + 125	+	Probiotic + Prebiotic
Safmannan®	250	+	Prebiotic
Challenged Chicks	0	+	Nonee
Nonee-Challenged Chicks	0	-	Nonee

[1]Safmannan® is obtained from primary culture and the purification of selected *Saccharomyces cerevisiae* proprietary strain sold by Phileo®. Microsaf® is a probiotic obtained fromPhileo®, contains *Bacillus amyloliquefaciens, Bacillus pumilus*, and *Bacillus licheniformis*. Envera Goplus® is *LactoBacillus* probiotic from Animal Care Envera.

[2]Challenge with *Clostridium perfringens* occurred on day 16 and 17 of the experiment. 3 ml oral gavage 10^7 CFU/ml per each challenged bird for all birds in challenged groups.

All treatments were fed the commercial corn-soy basal diet which includes the ingredients and nutrients that are listed in table 4.

Table 4.2 Ingredient and calculated composition of basal diet

Ingredient	Percentage
Corn	58.43
Soybean Meal	34.49
DL-Methionine	0.23
Lysine HCL	0.18
AV Blend	2.76
Limestone	1.56
BIOFOS[1]	1.54
Salts	0.51
Vitamins Premix[2]	0.25
Trace minerals Premix[3]	0.05
Calculated Nutrient Content (%)	
Protein	22
ME (Kcal/Kg)	3050
Crude Fat	5.32
Crude Fiber	2.63
AV phosphate	0.45
Calcium	0.95
AV Methionine	0.53
AV Met+Cys	0.83
AV Lysine	1.19
Sodium	0.22
Potassium	0.86
Chloride	0.39

[1]Mono-calcium phosphate

[2]Vitamins premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

[3]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

The rearing room temperature was regulated by the computer controlled building thermostat. Birds were observed daily with regard to the general flock condition, room temperature, lighting, water, feed, and other unanticipated events for the house, and mortality for all pens. And the study terminated at day 21. Weight of birds and feed were recorded at day 0, 16, 21 of the study to calculate the performance

parameters (Body weight, phase weight gain, phase feed to gain, cumulative feed to gain, cumulative feed to body weight, productivity index (PI), phase mortality percentage, and cumulative mortality percentage.

Statistical Procedures

Data was analyzed as a complete randomized block design using GLM procedure of SPSS. Blocks and treatments were used as fixed factors in the statistical model. Where significance was detected in the overall model for each dependent variable; means were separated using Duncan's multiple range test. Fore days 10 and 16 the Nonee-challenged and challenged control groups were combined since the actual challenge did not take place until day 16 and 17 of the experiment.

RESULTS AND DISCUSSION

The results from this study showed no significant effect on body weight or phase weight gain of the chickens at day 10 from adding Envera Goplus®, Microsaf® probiotics, or Safmannan® YCW prebiotic, as there were no significant differences between the feed additives groups and the control group in body weight or phase weight gain at day 10 of the experiment. Adding Safmannan® YCW prebiotic affected phase feed to gain and cumulative feed to body weight negatively significantly compared to control group as table (4.3) shows. Envera Goplus® + Safmannan® combination affected phase feed to gain and PI negatively significantly as well. While Envera goplus® improved cumulative feed to body weight ratio significantly compared to

Safmannan® group at day 10. Microsaf® group did not show differences in any performance parameter compared to control group, but it showed significant improvement in PI compared to Envera Goplus® + Safmannan® mixture group. In a study was conducted by Hashim *et al.,* (2017), adding purified and semi-purified YCW to broilers' feed did not affect body weight significantly compared to control groups. Neither significant differences in FCR or PI between the groups at day 10 shown in same study. M'Sadeq *et al.*, (2015) and Fowler *et al.,* (2015) documented that adding YCW to broiler feed did not affect the chickens' performance during the early phase of the growth, and that might because of the absence of the significant stressors they explained.

In table 4.4, results showed no significant differences between all groups in any of the performance parameters at day 14 of the experiment, and it could be because of the same reason which is the absence of stress factor to show the positive effects of the feed additives.

Table 4.3 The Effect of Adding the Treatments on Day 10 Performance

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	PI [1]	Phase Mortality %
Probiotic Products Group						
Microsaf® 500	260.5 ± 4.1	222.8 ± 3.9	1.01 ± 0.01 [ab]	1.18 ± 0.01 [ab]	221 ± 5 [a]	0 ± 0
Envera Goplus® 500	258.3 ± 3.3	220.5 ± 3.4	1.00 ± 0.01 [a]	1.18 ±0.01 [ab]	220 ± 3 [ab]	0 ± 0
Probiotic and Prebiotic Mix Group						
Envera goplus® 500+ Safmannan® 125	247.6 ± 4.5	210.3 ± 4.3	1.01 ± 0.01 [ab]	1.20 ±0.01 [b]	208 ± 4 [b]	0 ± 0
Prebiotic Group						
Safmannan® 250	258.0 ± 4.6	220.5 ± 4.5	1.02 ± 0.01 [b]	1.19 ±0.02 [b]	217 ± 6 [ab]	0 ± 0
Control Group (No Antibiotic Alternative Additives)						
Noneee-Challenged Control	260±2.6	223.6 ± 2.6	1.00 ± 0.004 [a]	1.16 ± 0.005 [a]	225 ± 3 [a]	0 ± 0

[a,b] Means within a row with no common superscript differ significantly (P<0.05)

± Standard Deviation

[1] PI= Productivity Index

Table 4.4 The Effect of Adding the Treatments on Day 16 Performance

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	Cumulative Feed to Gain	PI [1]	Phase Mortality %	Cumulative Mortality %
Probiotic Products Group								
Microsaf® 500	544.7±7.3	279.3± 4.5	1.25± 0.01	1.32± 0.02	1.25± 0.01	273±4	0 ± 0	0 ± 0
Envera Goplus® 500	546.7±6.8	283.1± 5.5	1.25± 0.01	1.32± 0.01	1.25± 0.01	274±4	0 ± 0	0 ± 0
Probiotic and Prebiotic Mix Group								
Envera goplus® 500+ Safmannan® 125	531.9±7.7	278.6± 3.8	1.25± 0.01	1.30± 0.01	1.25± 0.01	267±4	0 ± 0	0 ± 0
Prebiotic Group								
Safmannan® 250	547.1±8.4	283.5± 4.4	1.25± 0.01	1.30± 0.01	1.25± 0.01	274±5	0 ± 0	0 ± 0
Control Group (No Antibiotic Alternative Additives)								
Noneee-Challenged Control	545.6±6.6	280.1± 3.7	1.25± 0.004	1.32± 0.01	1.24± 0.04	275±4	0 ± 0	0 ± 0

± Standard Deviation

[1] PI= Productivity Index

At day 21, the results did not show any significant differences between all probiotics, prebiotics, and their mixture groups in body weight, weight gain, cumulative feed to body weight ratio, PI, and phase and accumulative mortality percentages as table (4.5) shows. Microsaf® group showed higher phase feed to gain and cumulative feed to gain ratios compared to Noneee-challenged group significantly. The results from this study agreed with the results from other studies like (Johnson *et al.,* 2020) as they couldn't find significant differences between YCW additive group at day 21 compared to control group when they added YCW prebiotic to broiler diet during challenging with *Clostridium perfringens*.

Mohamed *et al.,* (2022) showed that adding certain concentration of *Bacillus* probiotic to broiler chicken's diets enhanced body weight significantly compared to the control group, while adding the same probiotic in lower concentration did not affect the body weight significantly. Likewise, Actisaf® did not affect performance parameters values significantly at day 21. Fesseha *et al.,* (2021) found adding 4 g of *LactoBacillus* probiotic to the chicken's diet could improve body weight and FCR significantly compared to the control group at day 21, while adding the same probiotic to broiler chicken's diets in 2 g and 1 g concentrations did not affect the body weight at day 21 of the birds' age. Lacto*Bacillus* feed additive at 2 g concentration affected FCR negatively compared to 4 g *LactoBacillus* group and the control group as well. In current study, Envera Goplus® the *LactoBacillus* probiotic group affected phase feed to gain and accumulative feed to gain ratios negatively compared to Noneee-challenged control

group, but it did not have any other effect on body weight, weight gain, cumulative feed to body weight, mortality percentage at day 21 of the experiment.

Table 4.5 The Effect of Adding the Treatments on Day 21 Performance

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	Cumulative Feed to Gain	PI [1]	Phase Mortality %	Cumulative Mortality %
Probiotic Products Group								
Microsaf® 500	842 ± 12	293 ± 10	1.56 ± 0.02	1.58 ± 0.05 [b]	1.34 ± 0.01 [b]	275 ± 15	13 ± 7	8 ± 5
Envera Goplus® 500	849 ± 16	299 ± 11	1.55 ± 0.01	1.55 ± 0.05 [ab]	1.33 ± 0.01 [ab]	285 ± 9	9 ± 5	6 ± 3
Probiotic and Prebiotic Mix Group								
Envera goplus® 500+ Safmannan® 125	830 ± 18	291 ± 12	1.51 ± 0.02	1.48 ± 0.03 [ab]	1.31 ± 0.01 [ab]	301 ± 7	0 ± 0	0 ± 0
Prebiotic Group								
Safmannan® 250	853 ± 18	303 ± 16	1.51 ± 0.02	1.47 ± 0.05 [ab]	1.31 ± 0.01 [ab]	304 ± 10	3.3 ± 0	2.2 ± 0
Control Groups (No Antibiotic Alternative Additives)								
Challenged Chicks	828 ± 21	288 ± 10	1.53 ± 0.02	1.48 ± 0.02 [ab]	1.32 ± 0.01 [ab]	300 ± 8	0 ± 0	0 ± 0
Noneee-Challenged Control	871 ± 12	313 ± 6	1.51 ± 0.01	1.45 ± 0.02 [a]	1.30 ± 0.01 [a]	319 ± 5	0 ± 0	0 ± 0

[a,b] Means within a row with no common superscript differ significantly ($P<0.05$)

± Standard Deviation

[1] PI= Productivity Ind

CONCLUSION

The results from this study did not show significant enhancement of broiler chicken's performance in Actisaf®, Envera Goplus® probiotic groups, Safmannan® prebiotic group, neither Envera Goplus® + Safmannan® mixture group at day 10, 16, or 21 of the experiment while challenging with *Clostridium perfringens* pathogen. Studies showed different outcomes from adding probiotics and prebiotic products. In a study conducted by Hashim *et al.*, (2017), purified and semi-purified YCW groups did not show significant differences in body weight at day 21 compared to challenged control group, while semi-purified YCW showed significant decrease in body weight compared to Noneee-challenged control group. No significant differences in FCR or PI between YCW groups and the control groups were shown either at day 21. M'Sadeq *et al.*, (2015) and Fowler *et al.,* (2015) documented that adding YCW to broiler feed did not affect the chicken's performance during the early phase of the growth. In contrast of the results of the current study, some researchers suggested that adding YCW to broiler chicken's diets could alleviate the challenge impact on broiler chicken's performance in other studies (Hashim *et al.,* 2017; Fowler *at al.,* 2015; M'sadeq *et al.,* 2015; De Oliveira *et al.,* 2019; Knap et al., 2010). De Oliveira *et al.,* (2019) reported the positive effect of adding *Bacillus amyloliquefaciens* to broiler chicken's diets on body weight and FCR after challenge with *Eimeria maxima* and *Clostridium perfringens*. Ahmat *et al.,* (2021) reported that adding *Bacillus amyloliquefaciens* products to broiler chicken's diets led to body weight improvement at day 21. But the results from this study showed there were

no significant positive effect of adding prebiotics, probiotics, and their mixture to broiler chicken's diets at day 10, 16, 21 of chickens' age.

CHAPTER V

EVALUATION OF THE EFFECT OF SAFMANNAN® PREBIOTIC, ACTISAF®, ENVERA GOPLUS® PROBIOTICS, AND XPC® ON FULL TERM BROILER PERFORMANCE

INTRODUCTION

Finding effective natural growth promoters is the trend currently, following the banning of using antibiotics as growth promoters in broiler chicken production. Many alternatives to antibiotic growth promoters like probiotics, prebiotics, postbiotics have been examined to study their effects on broiler chicken's performance and diseases prevention or mitigation in poultry production (Perić *et al.,* 2009; Humam *et al.,* 2019; and Zolkiewics *et al.,* 2020). Probiotics are live microorganisms which used to improve intestinal balance, if they are given in adequate amount to the animal, to enhance the health of the host animal (FAO/WHO, 2002). Developing stable microflora in broiler chicken's intestine takes more than two months (Kabir, 2009), so that adding microbial additives or their metabolite could be a good approach to early enhancement of the intestinal microbiota and health in broilers (Yadav and Jha, 2019). Intestinal microbiota could provide nutritional compounds to the host animal as end products of fermentation or by secretion the products like short chain fatty acids (SCFAs), enzymes, amino acids, vitamins such as B and K, in addition some microflora could absorb ions, in addition of producing antibacterial substances which could reduce disease occurrence like organic acids, hydrogen peroxide, and bacteriocin (Taheri *et al.,* 2009). *LactoBacillus spp.* and

Saccharomyces cerevisiae yeasts could be used to produce probiotics (Park *et al.*, 2016). Using these probiotic microorganisms seemed to be a successful strategy to substitute antibiotics as growth promoters. In a study, feeding *Lactobacilli* probiotic to broiler chickens enhanced body weight and weight gain (Fesseha *et al.*, 2021).). Feeding *LactoBacillus* culture to broiler chickens showed FCR enhancement at day 21of the experiment (Chen *et al.,* 2017; and Bhogoju *et al.,* 2021). *LactoBacillus* positive effects on broiler chicken's performance could occur by improving intestinal villi permeability which leads to enhanced nutrients absorption; hence body weight enhancement occur (Pertiwi and Mahendra, 2021). *Lactobacilli* could be used antibiotic alternative through multiple mechanisms to inhibit pathogens, such as producing organic acids, producing hydrogen peroxide (H2O2), and producing bacteriocin (Taheri *et al.*, 2009). *Saccharomyces cerevisiae* could be used in poultry production as antibiotic alternative as probiotic, or its YCW segments or metabolites could be used as prebiotics or postbiotics (Ahiwe *et al.*, 2021). The live yeast could be used as probiotics in broiler chicken's diets to enhance mineral digestibility thus enhances weight gain (Gao *et al.*, 2008). Feeding YCW prebiotic could improve broiler chicken production through enhancing body weight and FCR (Santin *et al.*, 2001; Fowler *et al.*, 2015; and Hashim *et al.*, 2019). Prebiotics are defined as Nonee-digestible food ingredients that improve host health by stimulating the growth and/or activity of one or a limited number of selected beneficial bacteria in the intestine of the animal (Gibson and Roberfroid, 1995). Postbiotics are described and defined by International Scientific association of Probiotics and Prebiotics (ISAPP) as a "inanimate microorganisms and/or their components preparation that leads

to the host health benefit" (Salminen *et al.,* 2021). Adding postbiotic products to broiler chicken's diets could enhance body weight and FCR according to (Humam *et al.,* 2019). This enhancement in production could be because of improvement of intestinal health, as Danladi *et al.,* (2022) proved that adding postbiotic to broiler chicken's diets could improve villi height and decreased crypt depth significantly. On the other hand, diets containing more than 10% Distillers dried grains (DDGs) to broiler chickens could impact intestinal permeability negatively because of high fiber content in DDGs, so it could affect utilization of energy and nutrients in diets as result and decrease the productivity of the animals (Kim *et al.,* 2021). Feeding DDGs to broiler chickens, which contain high levels of polyunsaturated fatty acids could elevate the chance for oxidative stress and immune function changing in broiler chickens (Min *et al.*, 2015). Adding different kinds of feed additives to broiler chickens to maintain gut health was the purpose of this study.

Safmannan® YCW prebiotic and Actisaf® *Saccharomyces cerevisiae* probiotic from Phelio Lessafre, Envera Go® *LactoBacillus* probiotic from Envera® , and XPC® *Saccharomyces cerevisiae* postbiotic from Diamond V® were used in this experiment as feed additives to enhance performance of full term chickens while adding DDGs in the diet.

MATERIAL AND METHOD

The study was approved by the Texas A&M institutional Animal Care and Use Committee (IACUC 2014 - 0030). Birds were housed at Texas A&M University Poultry Research Center.

Experimental Design and General Procedure

Total of one day (960) Cobb-700 male broiler chicks were randomly distributed among 60 (3' x 6') floor pens (16 birds per pen and 2 replicates per block) in TAMU poultry farm building. A well-used pine shavings litter was used as bedding. The pens were equipped with hanging feeders and nipple drinkers. Six dietary treatments were assigned to 5 location blocks arranged from the East to West end of the rearing facility. All chicks were vaccinated with a 2x dose of coccidiosis vaccine (Coccivac®-B52, Merck Animal Health). Experimental treatments are shown in table (5.1). A control group which fed basal diet with no additive, Safmannan® 250 ppm, Envera Goplus® $2X10^6$, Envera Goplus® $2X10^5$ + Safmannan® 250 ppm, Actisaf® 250 ppm, and XPC® 1250 ppm.

Table 5. 1 Experimental treatments

Treatment Products[1]	**Concentration (ppm)**	**Antibiotic Alternative**
Safmannan®	250	Prebiotic
Actisaf®	250	Probiotic
Envera Goplus®	$2X10^6$ (500 ppm)	Probiotic
Safmannan®+ Envera Goplus®	125 + $2X10^5$ (500ppm)	Prebiotic + Probiotic
XPC®	1250	Postbiotic
Control	0	Noneee

[1]Safmannan® is obtained from primary culture and the purification of selected Saccharomyces cerevisiae proprietary strain sold by Phileo®. Actisaf® is a highly concentrated micro granule form of proprietary Saccharomyces cerevisiae live yeast from Phileo®. Envera Goplus® is *LactoBacillus* probiotic from Animal Care Envera. XPC® is a yeast culture *Saccharomyces cerevisiae* yeast grown on a media of processed grain by-products, roughage products, cane molasses, malt and corn syrup sold by Diamond V.

For this study, a 3-phase feeding program (starter, grower & finisher) was utilized on a full term 42-day trial. A basal corn-soy diet was formulated for each of the 3 feeding phases and then subdivided to create 6 different experimental treatment diets as described earlier in table (5. 1) Diets were formulated to contain 5% DDG's (starter), 8% DDG's (grower) and 10% DDG's (finisher).

Table 5. 2 Ingredient and calculated composition of Starter basal diet

Ingredient	**Percentage**
Corn	50.10
Soybean Meal	26.82
DDGs	5.00
DL-Methionine	0.31
Lysine HCL	0.33
L-Threonine 98%	0.10
Fat Blended	0.84
Limestone	1.35
BIOFOS[1]	1.57
Salts	0.28
Vitamins Premix[2]	0.25
Trace minerals Premix[3]	0.05
Calculated Nutrient Content (%)	
Protein	22.00
ME (Kcal/Kg)	2970

Table 5.2 Continued

Ingredient	Percentage
Crude Fat	3.73
Crude Fiber	2.69
AV phosphate	0.45
Calcium	0.90
AV Methionine	0.61
AV Met+Cys	0.90
AV Lysine	1.20
Sodium	0.16
Potassium	0.9
Chloride	0.29

[1]Mono-calcium phosphate
[2]Vitimin premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.
[3]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

Table 5. 3 Ingredient and calculated composition of grower basal diet

Ingredient	Percentage
Corn	60.34
Soybean Meal	24.78
DDGs	8.00
DL-Methionine	0.45
Lysine HCL	0.49
L-Threonine 98%	0.68
Fat Blended	2.61
Limestone	1.46
BIOFOS[1]	1.36
Salts	1.29
Vitamins Premix[2]	0.25
Trace minerals Premix[3]	0.05
Calculated Nutrient Content (%)	
Protein	20.50
ME (Kcal/Kg)	3100
Crude Fat	5.02
Crude Fiber	2.48
AV phosphate	0.40
Calcium	0.90
AV Methionine	0.53

Table 5.3 Continued

Ingredient	Percentage
AV Met+Cys	0.81
AV Lysine	1.07
Sodium	0.16
Potassium	0.84
Chloride	0.28

[1]Mono-calcium phosphate
[2]Vitimin premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.
[3]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

Table 5. 4 Ingredient and calculated composition of finisher basal diet

Ingredient	**Percentage**
Corn	67.14
Soybean Meal	16.94
DDGs	10.00
DL-Methionine	4.38
Lysine HCL	0.29
Fat Blend	2.33
Limestone	1.37
BioFOS[1]	1.17
Salts	0.10
Sodium Bicarb	0.25
Vitamins Premix[2]	0.25
Trace minerals Premix[3]	0.05
Calculated Nutrient Content (%)	
Protein	17.50
ME (Kcal/Kg)	3150
Crude Fat	5.00
Crude Fiber	2.42
AV phosphate	0.38
Calcium	0.80
AV Methionine	0.38

Table 5.4 Continued

Ingredient	Percentage
AV Met+Cys	0.62
AV Lysine	0.90
Sodium	0.16
Potassium	0.70
Chloride	0.17

[1]Mono-calcium phosphate
[2]Vitimin premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.
[3]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

Birds were observed daily with regard to general flock condition, temperature, water, feed, and any unanticipated events. Daily mortality was registered. Death of birds within the first 3 days of the study were replaced. The pen average was the unit of measure for the performance and histology analysis.

Weight of birds and feed were recorded at day 0. 21, 35, 42 of the experiment to calculate the performance parameters (body weight, phase weight gain, phase feed to gain, cumulative feed to gain, phase feed to body weight, Cumulative feed to body weight, productivity index (PI), phase mortality percentage and cumulative mortality. Performance parameters were evaluated on day-21, 35 and 42 of the rearing period.

Ten feces samples per pen pooled for dry matter at day 14, 28, 41. The samples dried in the lab to find the dry matter percentage in feces of the birds.

Histology Samples Preparation

At day-21 of the trial 2 birds per pen were randomly selected from each pen for histopathological evaluation (villi height, crypt depth, and crypt: villus ratio). A 3 cm ilium section was taken from each bird. The ileum was defined as that portion of the small intestine extending 20 mm from the vitelline diverticulum to a point 20 mm proximal to the ileo-caecal junction. Intestinal samples were fixed in formalin for further analysis.

Statistical Procedures

Collected data were analyzed by feeding phase as a one-way ANOVA using the GLM procedure of SPSS. Treatment and block were used as fixed factors. If significance was detected means were separated by Duncan's multiple range test. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Performance results from the three phases (starter, grower, and finisher) are shown in table (5. 5), (5. 6), (5. 7), and table (5. 8). Table (5. 9) shows dry matter percentage in fecal samples at day 14, 28, and day 41. Table (5. 10) shows histology results at day 21 of the experiment.

At day 21, there were no significant differences between all groups in body weight or phase weight gain ratio. In table (5.5) cumulative feed to gain and phase feed to gain ratios did not show any significant differences between all additive groups, neither difference was shown between the additive groups and the control group. Despite

of the results from other studies which proved the positive effect of adding Lactobacilli probiotic, *Saccharomyces cerevisiae* probiotic, YCW prebiotic, and *Saccharomyces cerevisiae* postbiotic on broilers' body weight and FCR (Fesseha *et al.*, 2021, Chen *et al.*, 2017; Bhogoju *et al.*, 2021; Gao *et al.*, 2008; Santin *et al.*, 2001; Fowler *et al.*, 2015; and Hashim *et al.*, 2019; and Humam *et al.*, 2019), the current study proved the opposite at day 21 of the experiment, as no significant differences between all groups were shown in all performance parameters. The lack of stress factor could show the differences in the performance of feed additives groups compared to the control group, as in spite of adding DDGs to the starter diet in 5%, it was not a high percentage to cause the damage to the intestine.

Table 5. 5 Growth and Performance at day 21 of the experiment

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	PI [1]	Phase Mortality%
Probiotic Products Group						
Actisaf®	792 ± 22	755 ± 22	1.38 ± 0.02	1.45 ± 0.03	252 ± 18	3.1 ± 5.3
Envera Goplus®	792 ± 37	755 ± 37	1.41 ± 0.06	1.48 ± 0.07	253 ± 20	1.3 ± 2.6
Probiotic and Prebiotic Mix Group						
Envera Goplus® + Safmannan®	792 ± 51	755 ± 501	1.39 ± 0.13	1.46 ± 0.15	247 ± 40	5.6 ± 8.0
Prebiotic Group						
Safmannan®	780 ± 39	742 ± 39	1.41 ± 0.02	1.48 ± 0.02	237 ± 21	5.6 ± 5.5
Postbiotic Group						
XPC®	790 ± 45	753 ± 45	1.41 ± 0.06	1.49 ± 0.07	234 ± 41	8.8 ± 9.9
Control Group (No Antibiotic Alternative Additive)						
Control Group	800 ± 35	763 ± 35	1.41 ± 0.03	1.49 ± 0.03	250 ± 17	2.5 ± 4.4

± Standard deviation

[1] PI = Productivity Index

At the second phase of the experiment (day 21-35), there were no significant differences between all groups in body weight or phase weight gain as table (5.6) shows. However, Actisaf®, and the combination of Envera Goplus® + Safmannan® groups showed significant improvement in cumulative feed to body weight and cumulative feed to gain ratios compared to the control group. Phase feed to gain ratio showed significant enhancement in Actisaf®, Envera Goplus®, and the combination of Envera Goplus® + Safmannan® groups compared to control group. PI and mortality percentage values of all groups weren't significantly different from each other as table (5.6) displays. These results agreed with some other studies as they showed that adding *Saccharomyces cerevisiae* probiotic to broiler diet enhanced feed digestibility (Gao *et al.*, 2008) which could be the reason to enhance feed to gain and feed to body weight ratios. Enhancing intestinal microbiota and intestine health could be the reason to enhance phase feed to gain in Envera Goplus® group as it contains *Lactobacilli* as (Yadav and Jha, 2019) stated that *Lactobacilli* has the role to improve the intestinal health of the animal. Additionally, the improvement occurred in cumulative feed to body weight and cumulative feed to gain ratios of Envera Goplus® + Safmannan® group could be referred to the enhancement in intestinal health which lead to better nutrient absorption to be reflected on less feed consumption and more weight gaining.

Table 5. 6 Growth and Performance at day 35 of the experiment

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	Cumulative Feed to Gain	PI [1]	Phase Mortality %	Cumulative Mortality %
Probiotic Products Group								
Actisaf®	1938 ± 83	1146 ± 70	1.66 ± 0.03^{a}	1.87 ± 0.06^{a}	1.69 ± 0.04^{a}	316 ± 32	0.6 ± 2.0	3.8 ± 5.3
Envera Goplus®	1908 ± 117	1115 ± 102	1.68 ± 0.07^{ab}	1.90 ± 0.10^{a}	1.72 ± 0.07^{ab}	312 ± 25	0.6 ± 2.0	1.9 ± 3.0
Probiotic and Prebiotic Mix Group								
Envera Goplus® + Safmannan®	1946 ± 79	1154 ± 55	1.66 ± 0.07^{a}	1.87 ± 0.06^{a}	1.70 ± 0.07^{a}	309 ± 34	0.0 ± 0.0	5.6 ± 8.0
Prebiotic Group								
Safmannan®	1887 ± 79	1106 ± 61	1.69 ± 0.04^{ab}	1.93 ± 0.08^{ab}	1.73 ± 0.04^{ab}	292 ± 29	0.7 ± 2.1	6.3 ± 5.9
Postbiotic Group								
XPC®	1893 ± 87	1103 ± 46	1.69 ± 0.02^{ab}	1.94 ± 0.04^{ab}	1.73 ± 0.03^{ab}	266 ± 42	0.0 ± 0.0	8.8 ± 9.9
Control Group (No Antibiotic Alternative Additive)								
Control Group	1900 ± 107	1101 ± 80	1.73 ± 0.04^{b}	1.98 ± 0.06^{b}	1.76 ± 0.04^{b}	297 ± 27	1.3 ± 2.8	3.8 ± 6.0

^{a,b} Means within a row with no common superscript differ significantly ($P<0.05$)

$\pm$ Standard Deviation

[1] PI= Productivity Indx

At day 42, there were no significant differences between all additive groups and the control group in all performance parameters. As table (5.7) shows, there were no significant differences in body weight, weight gain, cumulative feed to body weight ratio, phase feed to gain, cumulative feed to gain ratio, PI, or mortality percentages between all groups that used in the current experiment. Some studies agreed with these results, like a study was performed by (Wulandari and Syahniar, 2019) which couldn't prove a significant effect of adding *Saccharomyces cerevisiae* to broiler chicken's diets on their performance. Nevertheless the positive effects of adding feed additives to animals' feed, the results lack consistency from plan to plan as Kim and Lillehoj (2019) stated. Some studies showed that adding yeast probiotic enhanced performance as Yasar and Yegen (2017) proved that adding *Saccharomyces cerevisiae* to broiler diet enhanced body weight significantly at day 21 and 42 of the experiment. Other studies proved the enhancement in broilers' performance while *Lactobacilli* probiotic was provided as feed additive (Fesseha *et al.,* 2021; Chen *et al.,* 2017; and Bhogoju *et al.,* 2021), as Fesseha *et al.*, (2021) found that adding 4 g of *lactobacilli* to broiler diet led to significant improvement in body weight at week 1 and 2, and the final body weight at 5 weeks compared to control group, while adding I g of *lactobacilli* to the diet caused enhancement in broilers' body weight at week 2 and 5, and the final body weight at week 5. Feeding *LactoBacillus* culture to broilers showed FCR enhancement (Chen *et al.,* 2017; and Bhogoju *et al.,* 2021). There are studies proved YCW prebiotic efficiency in improving broiler chicken's performance, as they showed that feeding YCW prebiotic could improve broiler chicken

production through enhancing body weight and FCR (Santin *et al.,* 2001; Fowler *et al.,* 2015; Hashim *et al.,* 2019; and Humam *et al.,* 2019), which it couldn't been proved in the current study. The reason could be the absence of stress factor since the DDGs percentage did not exceed 10% in all diets which did not harm the intestinal tract of the animals.

Table 5. 7 Growth and Performance at day 42 of the experiment

Treatment	Body Weight (g)	Phase Weight Gain (g)	Cumulative Feed to Body Weight	Phase Feed to Gain	Cumulative Feed to Gain	PI [1]	Phase Mortality %	Cumulative Mortality %
Probiotic Products Group								
Actisaf®	2584 ± 127	646 ± 96	1.76 ± 0.03	2.12 ± 0.15	1.79 ± 0.04	330 ± 38	0.8 ± 2.6	4.4 ± 6.6
Envera Goplus®	2501 ± 144	594 ± 68	1.78 ± 0.06	2.17 ± 0.15	1.81 ± 0.06	321 ± 25	0.7 ± 2.3	2.5 ± 3.2
Probiotic and Prebiotic Mix Group								
Envera Goplus®+ Safmannan®	2575±130	629 ± 64	1.78± 0.04	2.18 ± 0.09	1.81 ±0.04	319 ± 36	0.8 ± 2.6	6.3 ± 8.8
Prebiotic Group								
Safmannan®	2491 ± 150	605 ± 102	1.80 ± 0.04	2.22 ± 0.28	1.84 ± 0.05	304 ± 40	0.0 ± 0.0	6.3 ± 5.9
Postbiotic Group								
XPC®	2522 ± 152	629 ± 107	1.80 ±0.05	2.22 ± 0.26	1.84 ± 0.05	299 ± 43	0.0 ± 0.0	8.8 ± 9.9
Control Group (No Antibiotic Alternative Additive)								
Control Group	2559 ± 220	659 ± 156	1.81 ± 0.06	2.13 ± 0.25	1.84 ± 0.06	318 ± 31	0.0 ± 0.0	3.8 ± 6.0

± Standard deviation

[1] PI = Productivity Index

Table (5. 8) displays the results of dry fecal matter analysis. There were no significant differences in fecal dry matter percentage between all six groups in all collection days, day 14, day 28, and day 41 of the experiment. The reason could be the absence of the stress factor as mentioned before, as the positive effect of probiotic, prebiotic, or symbiotic will be more vivid in the presence of stress.

Table 5. 8 Fecal Dry Matter Percentage

Treatment	Day 14	Day 28	Day 41
Probiotic Products Group			
Actisaf®	23.63 ± 5.47	19.54 ± 1.35	18.61 ± 2.20
Envera Goplus®	21.85 ± 1.49	18.77 ± 1.91	18.64 ± 1.59
Probiotic and Prebiotic Mix Group			
Envera Goplus® + Safmannan®	21.97 ± 2.09	18.44 ± 1.90	19.56 ± 1.39
Prebiotic Group			
Safmannan®	22.25 ± 2.44	19.35 ± 1.16	18.73 ± 0.78
Postbiotic Group			
XPC®	24.83 ± 5.75	19.48 ± 1.28	18.57 ± 1.85
Control Group (No Antibiotic Alternative Additive)			
Control Group	23.31 ± 2.37	20.78 ± 5.48	17.84 ± 2.26

± Standard deviation

Histology results are displayed in table (5.9). There were no significant differences in villi height, crypt depth, or V:C ratio in this experiment at day 21 of the animals' age. A study was conducted by Abdaljaleel *et al.,* (2018) showed there was no significant effect of adding YCW to broiler chicken's diets on villi height or crypt depth.

In contrast of these results, Qiu *et al.,* (2022) found that adding probiotic product to broiler chicken's diets enhanced intestinal morphology by improving villi height and V:C ratio significantly, in addition to decrease crypt depth significantly. Danladi *et al.,* (2022) proved that villi height increased significantly while crypt depth decreased significantly when postbiotic product was added to broilers' diet. Morales-López *et al.,* (2009) found that adding YCW to broiler chicken's diets, significantly increased villi height compared to control group.

Table 5. 9 Histology at day 21 of the experiment

Treatment	**Villi Height (µm)**	**Crypt Depth (µm)**	**V: C**
Probiotic Products Group			
Actisaf®	1263 ± 195	190 ± 109	7.49 ± 2.15
Envera Goplus®	1203 ± 135	174 ± 27	7.07 ± 1.22
Probiotic and Prebiotic Mix Group			
Envera Goplus®+ Safmannan®	1176 ± 196	178 ± 32	6.79 ± 1.56
Prebiotic Group			
Safmannan®	1238 ± 115	182 ± 34	7.02 ± 1.37
Postbiotic Group			
XPC®	1256 ± 207	166 ± 21	7.73 ± 1.90
Control Group (No Antibiotic Alternative Additive)			
Control Group	1215 ± 168	179 ± 33	7.01 ± 1.67

± Standard deviation

CONCLUSION

Adding different feed additives to broiler chicken's diet showed different effects on broiler chicken's performance in three rearing phases from day 1 to day 42 of birds' age. There were no significant differences between all groups in performance parameters at day 21 of the experiment. Groups contains live microorganisms like Actisaf®, Envera Goplus®, and the combination of Envera Goplus® + Safmannan® showed significant enhancements in phase feed to gain ratio compared to control group at day 35 of the experiment. Furthermore, Actisaf® group and the combination of Envera Goplus® + Safmannan® group showed significant improvement in cumulative feed to body weight and cumulative feed to weight gain ratios compared to control group at day 35 of the experiment. At day 42, no significant differences in performance parameters were detected between all groups in the current experiment. Additionally, there were no significant differences between all groups in fecal dry matter percentage at day 14, 28, and 41 of the experiment. There were no differences between all groups in in intestinal morphology at day 21, as there were no significant differences between villi height, crypt depth, V:C ratio between all groups. Some studies agreed to these results as they suggested that adding probiotic, YCW prebiotic to broiler diet did not enhance broilers' performance (Wulandari and Syahniar, 2019 and Hashim *et al.,* 2017). A study was performed by (Abdaljaleel *et al.,* 2018) suggested that adding YCW prebiotic did not affect villi height or crypt depth significantly, but it affected villi width. Other researcher suggested the opposite, as they found that adding probiotic, prebiotic, or postbiotic could enhance broiler performance (Fesseha *et al.,* 2021; Chen *et al.,* 2017; Bhogoju *et al.,* 2021; Santin *et al.,* 2001; Fowler

et al., 2015; Hashim *et al.,* 2019; Humam *et al.,* 2019). This variation in results could be attained by the variation of stressors presence and severity and the management.

CHAPTER VI

EVALUATION OF MIXTURE OF DIFFERENT LEVELS OF SAFMANNAN® PREBIOTIC AND *BACILLUS* PROBIOTIC ON BIRDS SUBJECTED TO BURSA VACCINE AND *CLOSTRIDIUM PERFRINGENS* CHALLENGE

INTRODUCTION

Poultry production could experience economical loses because of necrotic enteritis, mainly after banning the sub-therapeutic antimicrobial usage in poultry feed as growth promoters by many countries (Abd El-Hack *et al.,* 2021). *Clostridium perfringens* is the most significant cause of necrotic enteritis, and the high rate of adhesion of *Clostridium perfringens* to the intestinal mucosa cause necrotic enteritis and ulcerative enteritis, which could be clinical or subclinical (Songer, 1996; Williams, 2005). The clinical necrotic enteritis causes; diarrhea, anorexia, ruffled feathers, depression and sudden death, while the subclinical type of necrotic enteritis is characterized by reduction in production, no or shallow diarrhea, and low mortality (Freedman *et al.,* 2015; and Van Immerseel *et al.,* 2009). *Clostridium perfringens* can inhibit birds and cause necrotic enteritis combined with other influencing factors such as physical damage of intestine by feed or other infections like *Coccidia* or *Eimeria* (Williams, 2005; Petit *et al.,* 1999: Immerseel *et al.,* 2004). Type of feed could affect the severity of necrotic enteritis occurrence, as feeding pellet instead of mash diet could lower *Clostridium perfringens* count in intestine of the birds (Engberg *et al.,* 2002). The

type of feed of the broiler chickens could affect the wellbeing of intestine and necrotic enteritis occurrence, as the results from a study showed lower mortality rate in animals fed corn diet compared to animals fed wheat, rye or barley while contamination with *Clostridium perfringens* (Riddell and Kong, 1992). Immunosuppression has the impact to increase *Clostridium perfringens* outbreaks chances in birds as well, which could happen followed a primary infection, such as chicken anemia virus, infectious bursal disease, or Marek's disease. (Williams, 2005). Because antibiotics are currently prevented from being used in animal farms unless they are used as therapeutic drugs under supervision of licensed veterinarian (FDA, 2022), antibiotic alternatives have been used to enhance the broiler chicken's health through treating subclinical infections and improving production as well (Gadde *et al.*, 2017). Beneficial microorganisms have been used as probiotics in poultry (Kabir, 2009). Probiotics are defined as mono or mixed cultures of live organisms which enhance the animal's health if administered in a sufficient amount (FAW/WHO, 2001). *Bacillus* products has been used widely as antibiotic alternatives because of the highly tolerance of these bacteria to high temperatures and acidic pH, as they reach to the intestine of the animal without damage due to the low stomach pH and high body temperature of the chicken (Patlan *et al.*, 2019). Studies showed that feeding *Bacillus subtilis* to broiler chickens could improve growth performance, nutrient digestibility, immune response, and cecal microflora (Boroojeni *et al.*, 2018; Mohamed *et al.*, 2022). In addition of enhancing the production, adding *Bacillus subtilis* to broiler chicken's diets could decrease *Clostridium perfringens* count in caca (Qiu *et al.*, 2021). *Bacillus amyloliquefaciens* could improve FCR and

lower mortality percentage in case challenging with *Eimeria maxima* and *Clostridium perfringens* compared to challenge group without probiotic additive (Oliveira *et al.,* 2019). Adding *Bacillus pumilus* to broiler diet could enhance digestibility coefficient of protein, and it could improve body weight and feed intake (Pudova *et al.,* 2020; Bilal *et al.,* 2020). The outcomes from several studies suggested that dietary *Bacillus licheniformis* could be used in broiler chicken feed as alternative treatment to combat the negative effect of necrotic enteritis caused by *Clostridium perfringens* in poultry industry (Knap *et al.,* 2010). In addition of probiotics, prebiotics could be used as antibiotic alternative to enhance broiler performance. Prebiotics as it was defined by (Gibson and Roberfroid, 1995) are Nonee-digestible food ingredients which improve host health by enhancing the growth and/or activity of one or a limited number of microflora in the intestine of the animal. Yeast cell wall (YCW) is a prebiotic that's used in broiler production to improve the performance as some studies proved this functionality, and this improvement in performance could be attributed to enhancement of feed efficiency, and that occurred because of enhancement in immune response to microbial challenge and improve the intestinal health after adding YCW to the diet (Morales-lopez and Brufau, 2013). Adding YCW to broiler chicken's diets could improve FCR significantly as it improves villi height in intestine (Pascual *et al.,* 2020). Intestinal health and immunity could be enhanced by adding YCW to broiler chicken's feed while challenging with *Clostridium perfrengins,* and as a result, improvement of performance of the chickens occurs (Caly *et al.,* 2015; Fowler *et al.,* 2015). Adding YCW product to broiler chicken's feed could lower ileal *Clostridium perfringens* count

(Abudabos and Yehia, 2013). Synbiotics, which was first introduced in 1995 by Gibson and Roberfroid, are combination of a probiotic and a prebiotic. International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2019 defined symbiotic as a mixture that consist of live microorganisms and substrate(s) which selectively utilized by host microflora and lead to host's health benefit. For this purpose, this study was conducted to study the effect of adding different concentrations of *Bacillus* probiotic and YCW prebiotic mixtures to broilers' diet on broilers chicken's performance while challenging with *Clostridium perfringens*.

MATERIAL AND METHOD

The study was approved by the Texas A&M institutional Animal Care and Use Committee (IACUC 2014 - 0030) and the Animal Care and Use Committee at the Southern Plains Agriculture Research Center**.** Birds were housed at the Southern Plains Agricultural Research Center, United States Departments of Agriculture.

Experimental Design and General Procedure

A total of 288 Cobb-500 broiler chicks were procured and randomly distributed between two stainless steel battery units (48 pens). A total of 6 replicates (6 birds per pen) were sequentially assigned to pens such that each treatment was represented at least once for any given level of pens (4 levels). In this experiment, Safmannan® prebiotic was used in two concentrations (125 ppm and 250 ppm) in the diet of the treatment groups. *Bacillus* probiotic were used in three levels (10^4, 10^5, and 10^6). Eight groups were

used in this experiment as explained as following: Challenged control was challenged control with *Clostridium perfringens* and no additive to the basal feed, Noneee-challenged control was a not challenged with *Clostridium perfringens* and no feed additive was added to the basal diet, FB4 + Safmannan® 125 group which was challenged with *Clostridium perfringens* and fed basal diet in addition to 125 ppm Safmannan® $+10^4$ *Bacillus*, FB4 + Safmannan® 250 group, was challenged with *Clostridium perfringens* and fed 250 ppm Safmannan® $+10^4$ *Bacillus*, FB5 + Safmannan® 125 group which was challenged with *Clostridium perfringens* and fed 125 ppm Safmannan® $+10^5$ *Bacillus*, FB5 + Safmannan® 250 group which was challenged with *Clostridium perfringens* and fed 250 ppm Safmannan® $+10^5$ *Bacillus*, FB6 + Safmannan® 125 group which was challenged with *Clostridium perfringens* and fed 125 ppm Safmannan® $+10^6$ *Bacillus*, and FB6 + Safmannan® 250 group which was challenged with *Clostridium perfringens* and fed 250 ppm Safmannan® $+10^6$ *Bacillus*. Table (6.1) explains the feed additives and the challenge use in each group.

Table 6. 1 Experimental treatments

Treatment Products[1]	**Safmannan® Concentration (ppm)**	**FB (10^x)**	***Clostridium perfringens* Challenge**[2]
FB4 + Safmannan® 125	125	10^4	+
FB4 + Safmannan® 250	250	10^4	+
FB5 + Safmannan® 125	125	10^5	+
FB5 + Safmannan® 250	250	10^5	+
FB6 + Safmannan® 125	125	10^6	+
FB6 + Safmannan® 250	250	10^6	+
Challenged Control	0	0	+
Noneee-Challenged control	0	0	-

[1]Safmannan® is obtained from primary culture and the purification of selected Saccharomyces cerevisiae proprietary strain sold by Phileo®. FB is a *Bacillus* probiotic from Phileo®.

[2]Challenge with *Clostridium perfringens* occurred on day 16 and 17 of the experiment. 3 ml oral gavage 10^7 CFU/ml per each challenged bird for all birds in challenged groups.

The rearing room temperature was regulated by the computer controlled building thermostat. Fluorescent 48-inch tubes were used to provide 24-h constant light. At day-10 of the trial, one bird per pen was removed for necropsy and rest of the birds were vaccinated with a commercial Infectious Bursa Disease vaccine except birds in the Nonee-challenged control group. At day-16 one bird per pen was removed for necropsy and rest of the birds were challenged with *Clostridium perfringens* (3 ml oral gavage 10^7 CFU/ml) except birds in the Noneee-challenged control group. The

Clostridium perfringens challenge was repeated on day-17. Four birds were remain in each pen by the end of the trial. No antibiotics or coccidiostats were used in this experiment. Chickens received standard vaccinations at the hatchery.

Experimental diets were fed as crumbled and were stored in plastic containers throughout the trial. The first diet was formulated as a practical corn-soy based diet (D1). The second basal diet (D2) was formulated containing wheat middling and distiller's dried grains (DDG's). Both basal diets were divided into 7 equally-sized portions to create a total of 7 dietary treatments as control diet and six different treatment diets. Feed D1 was offered from 0-13 days of the chickens' age. Feed D2 was offered from 14-21 days of the chicken's age.

Table 6. 2 Ingredient and calculated composition of Diet 1 basal diet

Ingredient	**Percentage**
Corn	58.43
Soybean Meal	34.49
DL-Methionine	0.23
Lysine HCL	0.18
AV Blended	2.76
Limestone	1.56
BIOFOS[1]	1.54
Salts	0.51
Vitamins Premix[2]	0.25
Trace minerals Premix[3]	0.05

Calculated Nutrient Content (%)	
Protein	22.00
ME (Kcal/Kg)	3050
Crude Fat	5.32
Crude Fiber	2.63
AV phosphate	0.45
Calcium	0.95
AV Methionine	0.53
AV Met+Cys	0.83
AV Lysine	1.19
Sodium	0.22
Potassium	0.86
Chloride	0.39

[1]Mono-calcium phosphate

[2]Vitamins premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

[3]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

Table 6. 3 Ingredient and calculated composition of Diet 2 basal diet

Ingredient	Percentage
Corn	28.53
DDGs	6.41
Wheat	35.00
Soybean	22.97
DL-Methionine	0.26
Lysine HCL	0.31
L-Threonine 98%	0.07
Soybean Oil	3.20
Limestone	1.29
BIOFOS[1]	1.38
Salts	0.29
Vitamins Premix[2]	0.25
Trace minerals Premix[3]	0.05
Calculated Nutrient Content (%)	
Protein	19.00
ME (Kcal/Kg)	3086
Crude Fat	5.36
Crude Fiber	2.88
AV phosphate	0.42
Calcium	0.84

Table 6.3 Continued

Ingredient	Percentage
AV Methionine	0.47
AV Met+Cys	0.68
AV Lysine	0.99
Sodium	0.16
Potassium	0.84
Chloride	0.29

[1]Mono-calcium phosphate
[2]Vitimins premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin D3, 3,850 IU; vitamin E, 45.8 IU; vitamin B12, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin B6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.
[3]Trace minerals provided in the following, per kilogram of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg.

Water and feed were offered *ad libitum* throughout the entire trail period, and birds were observed daily with regard to the general flock condition, room temperature, lighting, water, feed, and other unanticipated events for the house, and mortality for all pens. Weight of birds and feed were recorded at day 0, 10, 14, 16, 21 of the experiment to calculate the performance parameters which included: body weight, phase weight gain, phase feed to gain ratio, cumulative feed to gain ratio, cumulative feed to body weight ratio, Productivity Index (PI), phase mortality percentage, and cumulative mortality percentage. The experiment was terminated at day 21 when all the feed and birds weights were taken before the birds were gassed using CO2.

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

Statistical Procedures

Data was analyzed as a complete randomized block design using the GLM procedure of SPSS. Blocks (battery level) and dietary treatments were used as fixed factors in the statistical model. When significance was detected in the overall model means was analyzed by Duncan's multiple range test. Significance was accepted at $P<0.05$

RESULTS AND DISCUSSION

The performance results were calculated at day 10, 14, 16, and 21 of the experiment. All results are displayed in the following tables, as each table presents one production parameter at a time with comparison between the groups at day 10, 14, 16, and 21.

Table (6.4) shows no differences in body weight between the groups at day 10, 14, or 16 of the age of the chickens. At day 21, FB4 + Safmannan® 250 group showed significant lower body weight compared to Noneee-challenged control at $P\leq0.05$. Table (6.5) shows phase weight gain results at day 10, 14, 16, and 21. There were no significant effect of the dietary additives on weight gain at day 10 or 14, while FB4 + Safmannan® 125 group showed significant lower phase weight gain compared to Noneee-challenged control at day 16, after vaccination with IBD vaccine and changing the diet to DDGs. At day 21, there were differences within the additive groups as FB4 + Safmannan® 125 group and FB4 + Safmannan® 250 group showed significant lower weight gain compared to FB6 + Safmannan® 250 group, which means the higher *Bacillus* and the higher YCW concentrations had the best effect on the combination to

enhance weight gaining of the birds at day 21, but there were no significant differences between additive groups compared to both challenged and Noneee-challenged control groups. Some studies supported these results as they did not show significant effect of adding prebiotic or probiotic to enhance broilers body weight. A study was conducted by Hashim *et al.,* (2017) showed adding YCW to broiler diet did not have significant effect to increase body weight at day 10. At day 16 of the same experiment, adding purified YCW to broiler chicken's diets did not have significant effect either, but adding semi-purified YCW to broiler chicken's diets caused significant decrease in body weight and weight gaining compared to control group. Purified YCW showed the same results at day 21 of that experiment, as there were no significant differences in body weight or weight gaining compared to challenge and Noneee-challenged groups with *Clostridium perfringens*, while the group fed with semi-purified YCW showed significant lower body weight and weight gaining compared to Noneee-challenged control only. In another study, they couldn't find significant improvement in broilers' body weight at day 10, 16, or 21 when YCW was added to the diet compared to control groups while challenging with *Clostridium perfringens* (Johnson et al., 2020). Pascual *et al.,* (2020) did not find significant differences in final body weight between YCW group and control group as well. Alkhulaifi *et al.,* (2022) did not find significant enhancement in daily weight gain when YCW was added to broiler chicken's diets while challenging with *Clostridium perfringens* compared to challenged group without YCW additive. Bilal *et al.,* (2020) collected the same results from adding *Bacillus* probiotic to broilers' diet, as they did not find significant differences in FCR values of *Bacillus* groups compared to

the control group between day 1 to 35 of the experiment. Ahmat *et al.,* (2021) found the opposite results from adding *Bacillus* probiotic to broilers chicken's diets, as they found significant enhancement in broiler chicken's body weight in *Bacillus* groups compared to control group. In another study, researchers could prove the significant effect of adding *Bacillus* to broiler chicken's diets on body weight at day 21, as the body weight was 1.465 lb to 1.596 lb in control group and *Bacillus* group respectively (Hooge *et al.*, 2004).

The reason of this variation in results could be attributed to the variation in concentration of the feed additives, as Mohamed[2] *et al.,* (2022) found that adding *Bacillus subtilis* to broilers' diet in 5 × 108 CFU/g concentration enhanced broilers' body weight compared to control group at day 21 of the experiment, while adding the same probiotic to broilers chicken's diets in lower concentration did not improve body weight of the broilers. Similarly, fowler *et al.,* (2015) suggested that the optimum concentration of YCW to add to broilers' diet is 250 ppm to acquire the positive effects on the broilers' performance.

Table 6. 4 DAY 10, 14, 16, and 21 Body Weight Results (g)

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	264 ± 9	468 ± 16	584 ± 23	910 ± 38^{ab}
FB4 + Safmannan® 250	256 ± 11	460 ± 16	581 ± 21	879 ± 62^{b}
FB5 + Safmannan® 125	257 ± 17	465 ± 24	591 ± 27	909 ± 53^{ab}
FB5 + Safmannan® 250	269 ± 13	469 ± 22	595 ± 32	931 ± 96^{ab}
FB6 + Safmannan® 125	265 ± 30	480 ± 35	603 ± 30	946 ± 48^{ab}
FB6 + Safmannan® 250	261 ± 12	466 ± 18	590 ± 23	943 ± 48^{ab}
Challenged Control	263 ± 8	466 ± 21	590 ± 23	906 ± 59^{ab}
Noneee-Challenged Control	273 ± 21	485 ± 23	614 ± 27	961 ± 32^{a}

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$)

± Standard deviation

Table 6. 5 DAY 10, 14, 16, and 21 Phase Weight Gain Results (g)

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	219 ± 10	198 ± 10	116 ± 9^{b}	314 ± 17^{b}
FB4 + Safmannan® 250	211 ± 12	196 ± 9	121 ± 7^{ab}	284 ± 54^{b}
FB5 + Safmannan® 125	212 ± 17	199 ± 11	126 ± 7^{ab}	302 ± 51^{ab}
FB5 + Safmannan® 250	224 ± 13	198 ± 6	125 ± 11^{ab}	326 ± 67^{ab}
FB6 + Safmannan® 125	220 ± 30	207 ± 6	123 ± 5^{ab}	331 ± 36^{ab}
FB6 + Safmannan® 250	216 ± 13	198 ± 7	124 ± 6^{ab}	345 ± 29^{a}
Challenged Control	218 ± 8	201 ± 10	124 ± 5^{ab}	312 ± 49^{ab}
Noneee-Challenge Control	228 ± 21	208 ± 10	129 ± 6^{a}	341 ± 24^{ab}

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$)

± Standard deviation

There were no significant differences in phase feed to weight gain ratio between the groups at day 10, as table (6.6) shows. At day 14, FB4 + Safmannan® 125, FB4 + Safmannan® 250, FB5 + Safmannan® 125, and FB5 + Safmannan® 250 groups showed significant higher feed to weight gain ratio compared to the challenged control group after subjecting to IBD vaccine and before challenging with *Clostridium perfringens*. At day 16, FB4 + Safmannan® 125 group showed lower phase feed to weight gain ratio significantly compared to both control groups in addition to FB5 + Safmannan® 250. At day 21, there were no significant differences between all groups in phase feed to weight

gain ratio. In table (6.7), there were no significant effect of the feed additives on cumulative feed to weight gain ratio at day 10 of the experiment, as there were no significant differences between all groups. At day 14, all the feed additive groups showed higher cumulative feed to weight gain ratio compared to control groups, and that was after IBD vaccination. FB4 + Safmannan® 125 and FB4 + Safmannan® 250 groups showed the same significant elevation in cumulative feed to weight gain ratio compared to both control groups. While at day 21, FB4 + Safmannan® 250 showed higher cumulative feed to weight gain significantly compared to Noneee-challenged group. Table (6.8) showed there were significant differences in cumulative feed to body weight at day 10 only, while there were no significant differences between all groups at day 14, 16, 21 of the experiment in cumulative feed to body weight. Hashim *et al.*, (2017) got the same results from adding purified YCW to broiler chicken's diets, they couldn't find significant effect on phase feed to gain, cumulative feed to gain, or FCR at day 10, 16, and 21 of the experiment, while adding semi-purified YCW to broiler chicken's diets impacted phase and cumulative feed to weight gain negatively compared to challenged and Noneee-challenge control groups significantly at day 16. Johnson *et al.*, (2020) did not find improvement in conversion ratio at day 10, 16, or 21 when YCW was added to broilers' diet, except phase weight to gain ratio of YCW group showed significant enhancement at day 16 compared to control group. Bilal *et al.*, (2020) did not find significant effect of adding *Bacillus* to broiler chicken's diets on FCR compared to control group between day 1 to 35. In a study, adding *Bacillus* probiotic to broiler chicken's diets did not affect FCR value significantly in another study which was

conducted by Ahmat *et al.,* (2021). Contrary to these results, other researchers suggested that adding YCW to broiler chicken's diets could enhance chicken's performance as it enhances FCR because of the intestine health improvement (Pascual *et al.,* 2020; and fowler *et al.,* 2015). ALkhulaifi *et al.,* (2022) as well, found adding 0.5 g/kg of YCW to broilers' diet enhanced FCR at day 25 of the chicken's age significantly while challenging with *Clostridium perfringens*. Hooge *et al.,* (2004) found adding *Bacillus subtilis* probiotic to broiler feed improved FCR significantly compared to control group at day 21 of the experiment.

Table 6. 6 DAY 10, 14, 16, and 21 Phase Feed to Weight Gain Ratio Results

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	1.18 ± 0.2	1.20 ± 0.04^{b}	1.43 ± 0.06^{b}	1.73. ± 0.10
FB4 + Safmannan® 250	1.19 ± 0.4	1.21 ± 0.03^{b}	1.39 ± 0.04^{ab}	1.82 ± 0.26
FB5 + Safmannan® 125	1.19 ± 0.3	1.21 ± 0.03^{b}	1.37 ± 0.08^{a}	1.74 ± 0.27
FB5 + Safmannan® 250	1.17 ± 0.01	1.21 ± 0.03^{b}	1.38 ± 0.05^{ab}	1.78 ± 0.25
FB6 + Safmannan® 125	1.19 ± 0.06	1.19 ± 0.04^{ab}	1.39 ± 0.03^{ab}	1.71 ± 0.18
FB5 + Safmannan® 250	1.19 ± 0.03	1.20 ± 0.04^{ab}	1.35 ± 0.04^{a}	1.63 ± 0.11
Challenged Control	1.15 ± 0.02	1.16 ± 0.03^{a}	1.37 ± 0.04^{a}	1.77 ± 0.21
Noneee-Challenged Control	1.17 ± 0.03	1.18 ± 0.03^{ab}	1.36 ± 0.05^{a}	1.63 ± 0.12

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$)

± Standard deviation

Table 6. 7 DAY 10, 14, 16, and 21 Cumulative Feed to Weight Gain Ratio Results

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	118 ± 0.02	1.19 ± 0.01[b]	1.24 ± 0.01[c]	1.38 ± 0.02[ab]
FB4 + Safmannan® 250	1.19 ± 0.04	1.20 ± 0.01[b]	1.24 ± 0.02[c]	1.40 ± 0.06[b]
FB5 + Safmannan® 125	1.19 ± 0.03	1.20 ± 0.03[b]	1.23 ± 0.04[bc]	1.38 ± 0.07[ab]
FB5 + Safmannan® 250	1.17 ± 0.01	1.19 ± 0.01[b]	1.23 ± 0.01[bc]	1.37 ± 0.02[ab]
FB6 + Safmannan® 125	1.19 ± 0.06	1.19 ± 0.2[b]	1.23 ± 0.02[bc]	1.37 ± 0.04[ab]
FB6 + Safmannan® 250	1.19 ± 0.3	1.20 ± 0.02[b]	1.23 ± 0.02[bc]	1.35 ± 0.03[ab]
Challenged Control	1.15 ± 0.02	1.15 ± 0.01[a]	1.20 ± 0.02[a]	1.36 ± 0.03[ab]
Noneee-Challenged Control	117 ± 0.03	1.17 ± 0.01[a]	1.21 ± 0.02[ab]	1.34 ± 0.04[a]

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$)

± Standard deviation

Table 6. 8 DAY 10, 14, 16, and 21 Cumulative Feed to Body Weight Ratio Results

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	0.978 ± 0.01^{b}	1.17 ± 0.02	1.22 ± 0.02	1.57 ± 0.04
FB4 + Safmannan® 250	0.982 ± 0.03^{b}	1.16 ± 0.04	1.20 ± 0.04	1.57 ± 0.07
FB5 + Safmannan® 125	0.980 ± 0.03^{b}	1.17 ± 0.03	1.21 ± 0.04	1.55 ± 0.09
FB5 + Safmannan® 250	0.975 ± 0.01^{ab}	1.18 ± 0.03	1.22 ± 0.03	1.58 ± 0.03
FB6 + Safmannan® 125	0.982 ± 0.03^{b}	1.17 ± 0.02	1.21 ±0.01	1.56 ± 0.04
FB6 + Safmannan® 250	0.987 ± 0.02^{b}	1.17 ± 0.02	1.21 ± 0.02	1.54 ± 0.04
Challenged Control	0.950 ± 0.02^{a}	1.15 ± 0.02	1.19 ± 0.01	1.57 ± 0.05
Noneee-Challenged Control	0.972 ± 0.02^{ab}	1.16 ± 0.02	1.20 ± 0.02	1.53 ± 0.05

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$)

$\pm$ Standard deviation

Productivity Index did not show differences at day 10 between all groups, as table (6.9) displays. FB4 + Safmannan® 250 showed lower PI value compared to both control groups at day 14, 16, and 21 significantly, while all other additive groups did not show differences in PI compared to challenged or Noneee-challenged control groups. Table (6.10) and table (6.11) showed no significant differences between groups in phase mortality percentage at day 10, 14, 16, and 21. Some studies proved the same results, as they couldn't prove significant a significant enhancement in PI or mortality percentage while adding YCW to broiler diet (Hashim *et al.,* 2017 and Johnson *et al.,* 2020). Qiu *et al.,*

(2021) found there were no significant differences in mortality percentages between the groups contains *Bacillus* probiotics and the control group, which explain the absence of *Bacillus* role in decrease mortality significantly. However, other studies proved positive effect from adding YCW to broilers' diet on broilers' performance, as they found adding YCW 0.5 g/kg of YCW to broiler diet significantly enhanced European production efficiency factor (EPEF) while challenging with *Clostridium perfringens* compared to the challenged group without YCW additive, furthermore, YCW group showed significant improvement in live ability percentage compared to challenged group without YCW additive.

Table 6. 9 DAY 10, 14, 16, and 21 PI[1] Results

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	223 ± 11	281 ± 10[ab]	295 ± 13[bc]	305 ± 26[ab]
FB4 + Safmannan® 250	209 ± 24	266 ± 23[b]	284 ± 22[c]	292 ± 43[b]
FB5 + Safmannan® 125	217 ± 14	278 ± 10[ab]	299 ± 10[abc]	315 ± 28[ab]
FB5 + Safmannan® 250	230 ± 13	282 ± 14[ab]	303 ± 17[abc]	297 ± 45[ab]
FB6 + Safmannan® 125	255 ± 36	289 ± 25[a]	307 ± 23[abc]	318 ± 35[ab]
FB6 + Safmannan® 250	219 ± 14	279 ± 11[ab]	301 ± 13[abc]	324 ± 37[ab]
Challenged Control	229 ± 8	289 ± 13[a]	308 ± 12[ab]	309 ± 33[ab]
Noneee-Challenged Control	235 ± 23	297 ± 14[a]	318 ± 14[a]	342 ± 16[a]

[a,b,c] Means within a row with no common superscript differ significantly ($P \leq 0.05$)
± Standard deviation
[1] PI= Productivity Index

Table 6. 10 DAY 10, 14, 16, and 21 Phase Mortality Percentage Results

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	0 ± 0	0 ± 0	0 ± 0	4.2 ± 10.2^{ab}
FB4 + Safmannan® 250	2.8 ± 6.8	0 ± 0	0 ± 0	0.0 ± 0.0^{a}
FB5 + Safmannan® 125	0 ± 0	0 ± 0	0 ± 0	0.0 ± 0.0^{a}
FB5 + Safmannan® 250	0 ± 0	0 ± 0	0 ± 0	12.5 ± 13.7^{b}
FB6 + Safmannan® 125	0 ± 0	0 ± 0	0 ± 0	5.0 ± 11.2^{ab}
FB6 + Safmannan® 250	0 ± 0	0 ± 0	0 ± 0	4.2 ± 10.2^{ab}
Challenged Control	0 ± 0	0 ± 0	0 ± 0	4.2 ± 10.2^{ab}
Noneee-Challenged Control	0 ± 0	0 ± 0	0 ± 0	0.0 ± 0.0^{a}

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$)
± Standard deviation

Table 6. 11 DAY 10, 14, 16, and 21 Cumulative Mortality Percentage Results

Treatment	Day 10	Day 14	Day 16	Day 21
FB4 + Safmannan® 125	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.8 ± 6.8
FB4 + Safmannan® 250	2.8 ± 6.8	2.8 ± 6.8	2.8 ± 6.8	2.8 ± 6.8
FB5 + Safmannan® 125	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FB5 + Safmannan® 250	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.3 ± 9.1
FB6 + Safmannan® 125	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 7.5
FB6 + Safmannan® 250	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.8 ± 6.8
Challenged Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.8 ± 6.8
Noneee-Challenged Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

± Standard deviation

CONCLUSION

Noneee of the combination additive enhanced broiler chicken's performance between day 1 to day 21 of the chickens age while challenging with *Clostridium perfringens* pathogen, as no improvement in total body weight, feed to body weight, feed to weight gain, PI, or mortality percentage between the additive groups while challenging with *Clostridium perfringens* and the challenged group without feed additive. This result could be attributed to the weakness of the challenge as there were no significant differences between the challenged and Noneee-challenged control

groups. The other reason of the differences absent between groups in performance could be the doses of the additives did not reach to the optimum threshold to show differences between groups, as scientists suggested that adding feed additives should be added in optimum concentration to show significant effects on performance or treating diseases (Mohamed[2] *et al.*, 2022; and Fowler *et al.*, 2015).

CHAPTER VII

ULTIMATE CONCLUSION

This research aimed to evaluate the effect of different types of feed additives (probiotics, prebiotics, postbiotics and their mixtures) on challenged or Noneee-challenged broilers with *Clostridium perfringens* or *Salmonella Typhimurium*, in starter and full term chickens performance. The additives in the certain concentrations that were used in five different studies didn't affect the broilers' performance significantly while challenging with *Salmonella Typhimurium* or *Clostridium perfringens* pathogens, neither there were significant effects of these feed additives on full term chickens performance.

As in the first study, postbiotics (XPC® 1250 ppm and XPC-Ultra® 625 ppm) and prebiotics (Safmannan® 125 ppm and Safmannan® 250 ppm) were added to broiler chickens' diet to evaluate their effects on the chickens performance while challenging with Salmonella Typhimurium. The results of this study didn't show significant enhancement in broilers' performance at day 10. Safmannan® 125 ppm showed significant reduction in *Salmonella Typhimurium* count on the plates compared to all other challenged groups. In the second study, postbiotics (XPC® 1250 ppm and XPC-Ultra® 625 ppm) and prebiotics (Safmannan® 250 ppm and Safmannan® 500 ppm)

were used to study their effects on broiler chickens performance while challenging with *Clostridium perfringens.* The results showed the additives groups didn't retain the performances to Noneee-challenged control-like at day 21, as they didn't enhance performance significantly compared to challenged control group with no additives. In the third study, Phelio Microsaf® probiotic 500 ppm, Envera Goplus® probiotic 500 ppm, Safmannan® prebiotic 250 ppm, and combination of Envera Goplus® 500 ppm + Safmannan® 125 ppm were used as feed additives in broilers' diet to study their effect on broiler performance while challenging with *Clostridium perfringens.* The outcomes from this study proved no significant enhancement in performance in additives groups compared to challenged control group at day 21. Envera Goplus® probiotic 500 ppm, Actisaf® probiotic 250 ppm, Safmannan® prebiotic 250 ppm, XPC® postbiotic 1250 ppm, and combination of Safmannan® 125 ppm + Envera Goplus® 500 ppm were used as feed additives to study their effect on full term broiler chickens performance, fecal dry matter percentage, and intestinal improvement in full term broilers' performance at day 42. No differences in fecal dry matter percentage or intestinal morphology among all groups either. The fifth study, showed no significant enhancement effect on broilers' performance when combinations of Safmannan® and *Bacillus* were added in different concentrations while challenging with *Clostridium perfringens.*

Some studies agreed with the results from these studies as they didn't find significant improvement in broilers' performance while adding probiotics, YCW products to broilers' diet like; (Wulandari and Syahniar, 2019; Hashim *et al.*, 2017; and Johnson *et al.*, 2020). Abdaljaleel *et al.*, (2018) didn't find significant enhancement in villi height or crypt depth while adding YCW to broilers' diet either. Although, Fesseha *et al.*, (2021), Chen *et al.*, (2017), Bhogoju *et al.*, (2021), Santin *et al.*, (2001), Fowler *et al.*, (2015), and Humam *et al.*, (2019) proved, that adding feed additives like probiotics, prebiotics, or postbiotics enhanced broilers' performance significantly. This difference in results between different studies could occur because of the variation of stressors severity and management, or the differences in feed additives concentrations that were used in different studies which cause lack of consistency of the outcomes after adding probiotics, prebiotics, postbiotics, or their combinations to broilers' diet as feed additives and antibiotic alternatives.

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