EFFECT OF A DIETARY PREBIOTIC AND PROBIOTIC ON BROILER PERFORMANCE AND CARCASS YIELD

by

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ABSTRACT

The expected forthcoming restrictions for the use of antibiotics as growth promoters (AGP) are a major concern for poultry producers. Another priority issue for the poultry industry is the control of foodborne pathogens at all stages of production. For this reasons, alternative strategies have to be adopted at the farm level to maximize bird's performance while maintaining a good health status. Previous research has suggested that the use of probiotics and prebiotics in feed could be an alternative to AGP and, at the same time, helping reduce the prevalence of foodborne pathogens in poultry digesta. The objective of this experiment was to determine the effect of a prebiotic and a probiotic on the productive performance and carcass yield of broiler chickens. A total of 600 1 d-old chicks were randomly distributed among 20 floor pens and were fed one of four dietary treatments from 1 – 41 d-of-age. The dietary treatments were as followed: Commercial Control (CON), CON + mannan-oligosaccharide prebiotic at a 0.1% inclusion (MOS), CON + Bacillus subtilis probiotic at a 0.05% inclusion (BAC), and CON + prebiotic + probiotic (M+B). Feed intake (FI), body weight (BW), and feed conversion ratio (FCR) were determined at 14, 28, 35, and 40 d-of-age. At 41 d, three males per pen were randomly selected and weighted for a carcass yield study. Three birds per pen were randomly selected, slaughtered, and ceca aseptically removed for microbiological analysis. Real-time PCR was used for detection of *Salmonella* and *Campylobacter* in ceca content. In a cumulative basis, FI at 41 d was higher (P=0.05) for birds fed CON and BAC, when compare to MOS. Despite FI behavior, no statistical differences were observed on BW or BW gain. At 28 d, broilers fed CON were more efficient than MOS, BAC, and M+B (P<0.01). However, by 35 and 40 d-of-age no differences in FCR were observed. For the carcass yield study, no significant differences were observed on live BW, carcass weight, or carcass yield. Pathogen analysis revealed the presence of *Campylobacter* in 12 of the total obtained samples, while *Salmonella* was absent in all samples. It was concluded that the MOS based prebiotic and the BAC

probiotic, at the inclusion levels studied, did not have a negative effect on broiler performance or carcass yield, while Campylobacter presence in ceca content was only present in a small number of the total pens. Future studies should concentrate on discovering an effective inclusion rate of these additives in diets to determine which inclusion rate is appropriate to increase performance levels in broilers.

Keywords: probiotics, prebiotics, broilers, food-borne pathogens

RESUMEN

Las futuras restricciones en el uso de antibióticos como promotores de crecimiento son una de las preocupaciones para los productores avícolas. Un tema de prioridad en la industria avícola es el control de patógenos de origen alimenticio en todas las etapas de producción. Por esta razón, estrategias alternas se deben comenzar a adaptar a nivel de finca para poder maximizar el desempeño de las aves. mientras se mantiene un buen estado de salud. Investigaciones previas han sugerido que el uso de prebióticos y probióticos en el alimento podría utilizarse como alternativa a los antibióticos y al mismo tiempo, contribuir en la reducción de la prevalencia de patógenos de origen alimenticio en la digesta de las aves. El objetivo en este experimento era determinar el efecto del uso de un prebiótico y un probiótico en el desempeño productivo y en el rendimiento de la carcasa de pollos parrilleros. Un total de 600 aves de 1 día de edad fueron aleatoriamente distribuidas en 20 jaulas, donde fueron alimentadas con uno de 4 tratamientos dietéticos desde 1 – 41 días de edad. Los tratamientos dietéticos fueron los siguientes: Control Comercial (CON), CON + el prebiótico mannan oligosacárido a una inclusión de 0.1% (MOS), el probiótico *Bacilus subtilis* a una inclusión de 0.05% (BAC), y CON + prebiótico + probiótico (M+B). En consumo de alimento (FI), peso corporal (BW), y la tasa de conversión alimenticia (FCR) fueron determinadas en los días 14, 28, 35 y 40 de edad. A los 41 días de edad, 3 machos de cada jaula fueron aleatoriamente seleccionadas y pesados para el estudio del rendimiento de carcasa. Tres aves por jaula fueron sacrificadas y los ciegos fueron removidos de manera aséptica para análisis microbiológico. La técnica de PCR en tiempo real fue utilizada para la detección de bacterias de los géneros *Salmonella* y *Campylobacter*. El FI acumulado a los 41 d fue mayor (P=0.05) para las aves alimentadas con CON y BAC, cuando se comparó con MOS. No se encontraron diferencias estadísticas en BW o ganancia de BW en ninguno de los periodos. A los 28 d, pollos parrilleros

alimentados con CON fueron más eficientes que MOS, BAC y M + B ($P<0.01$), pero a los 35 y 40 días de edad, ninguna diferencia en FCR fue observada. El estudio de rendimiento de carcasa no mostró diferencias significativas en el BW vivo, peso de carcasa o rendimiento de carcasa. El análisis de patógenos reveló la presencia de *Campylobacter* en 12 del total de muestras obtenidas, mientras que *Salmonella* estuvo ausente en todas las muestras. Se puede concluir que MOS y BAC no tuvieron efectos negativos en el desempeño de los pollos parrilleros o el rendimiento de carcasa, mientras que la presencia de *Campylobacter* solo afecto un pequeño número de la población de las muestras obtenidas. Futuros estudios deben concentrarse en descubrir una tasa de inclusión de estos aditivos en las dietas para poder determinar que tasa de inclusión es apropiada para aumentar el desempeño de los parrilleros.

Palabras clave: probióticos, prebióticos, pollos parrilleros, patógenos de origen alimenticio

Dedication

I dedicate this thesis to my mother Haydeé Vega and my brother Allan J. Elías, for being my pillar of strength and source of unconditional love.

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LIST OF ABBREVIATIONS

AdjFCR	**Adjusted Cumulative Feed Conversion Ratio**
BW	**Body Weight**
BWG	**Body Weight Gained**
C. jejuni	***Campylobacter jejuni***
CFCR	**Cumulative Feed Conversion Ratio**
CFI	**Cumulative Feed Intake**
FCR	**Feed Conversion Ratio**
FI	**Feed Intake**
WD	**Withdrawal**

1 INTRODUCTION

Due to concerns raised by the market, the general public and more recently by the USDA, the poultry industry is analyzing alternatives to antibiotics that are both economically feasible, and can maintain or improve performance. Among these alternatives probiotics were proved to favorably affect the animal's body by granting a balance in the intestinal microbial flora (Fuller, 1989).

While research has been done on the addition of probiotics and prebiotics in broiler diets (Chimote et al., 2009; Kim et al., 2011; Zhang et al., 2014), many do not fully prove its efficiency in broiler diets (Loh et al., 2014; Ghorbani et al., 2002**)**. In addition, many of these studies have been performed utilizing a Lactobacillus-based diet, as it is the most predominant microorganism in the poultry's intestinal flora. Lactobacilli have mechanisms that inhibit the growth and can kill several species of pathogenic bacteria (Fuller, 1989; Sarra and Badini, 1997).

As the chicken continues the intake of lactobacilli, this has a local immunostimulant effect on the intestinal mucosa, as Lactobacilli attract lymphocytes to the intestinal lamina propria (Lillehoj and Chung, 1992). This constant intake also stimulates the intestinal synthesis of IgA by the immune system, increasing the resistance to diseases (Pulverer et al., 1990).

Probiotics can be used as additives in poultry feed, as they selectively promote the growth of favorable species of bacteria, balancing the poultry's intestinal microflora. This study utilizes the probiotic *Bacillus subtilis*, a probiotic with the capability to proliferate in a wider range of intestinal environments. Bacterial spores are capable of adapting across the process of distribution and administration of animal feed, for example the pelleting process. These bacterial spores are also able to pass through the acidic gastric environment of the target host species as it enters the small

intestine in a viable state (Tactacan et al., 2013). A spore monoculture of *B. subtilis* has the capacity of being readily produced, capable of being stored for long periods of time and is avirulent. Many of these qualities make *B. subtilis* a suitable additive for the feed of production animals, including poultry (La Ragione et al., 2001).

"Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon" (Gibson and Manning, 2004). In this study *Mannan-oligosaccharide* (MOS), commonly found in the cell wall of the yeast (*Saccharomyces cerevisiae*), was utilized.

Mannose is the main component of MOS and because it is bound to the type 1 fimbriae used by many enteric bacteria to attach to host cells, it makes a suitable additive to promote the growth of beneficial bacteria (Kim et al., 2011). The mechanism of MOS will result in the movement of undesirable bacteria through the intestine without colonization (Newman, 1994), increase the production of IgA in laying hens (Kim et al., 2009), and dogs (Swanson et al., 2002 a,b). The increase of IgA will cause the inhibition of the attachment and penetration of bacteria in the lumen, increasing the population of mucus (McKay and Perdue, 1993), and prevents the inflammation that may cause epithelial tissue damage (Russell et al., 1989).

These feed additives (prebiotics and probiotics) have been supplemented to broilers resulting in an improved FCR. Thus it was hypothesized that the use of prebiotic and / or probiotic supplements (individually or combined) in a broiler's diet would also improve the intestinal microbiome and increase its intestinal nutrition absorption, which in combination will bring an improved broiler performance (Fuller, 1989; Sohail et al., 2011).

2 OBJECTIVE

The objective of this trial was to study the inclusion of a *Bacillus subtilis*-based probiotic and a mannanoligosaccharide (MOS)-based prebiotic alone or in combination on broiler performance, carcass yield, and presence of foodborne pathogens in ceca content.

3 LITERATURE REVIEW

3.1 *Salmonella*

Salmonella are part of the *Enterobacteriaceae* family. Varying among many serovars, these Gram-negative rod-shaped bacteria, are considered to be the leading foodborne pathogen, causing many illnesses in both farm animals and humans. (Andino and Hanning, 2015). Among the characteristics that many *Salmonella* serovars have in common is their ability to be lactose fermenters and to be producers of hydrogen sulfite, however there are other biochemical properties that permit the identification of *Salmonella* as well (Jensen and Hoorfar, 2000; Abulreesh, 2012). *Salmonella* serovars make the intestinal tract of humans and many animals such as poultry, to be their main niche. Since *Salmonella* remains colonizing in the gastrointestinal tracts, these are excreted in fecal matter, and contaminate feed, bedding, water, and soil, among other surfaces that make possible for *Salmonella* able to be transmitted to wild birds, reptiles and insects (Minor, 1992; Sanchez et al., 2002; Rodriguez et al., 2006).

Salmonellosis is a public health concern that causes great economical losses in the poultry industry. Among the many serovars that cause diseases in poultry*, Salmonella enterica serovar Gallinarum* (*S. gallinarum*) and *Salmonella enterica serovar Pullorum* (*S. pullorum)* are well known to cause fowl typhoid and the pullorum disease in poultry (Cheragchi et al., 2014). *Salmonella enterica serovar typhimurium* (*S. typhimurium*) is another serovar that causes great economical losses, but this pathogen causes intestinal inflammation that results in gastroenteritis. Both *S. gallinarum* and *S. pullorum* are non-motile, host adapted to poultry, but cause different in illnesses in poultry. Fowl typhoid can cause diarrhea, womb bleeding and kidney enlargement with an almost certain death rate this disease can cause high mortality rates in three to six months in production (Barrow et al., 2011; Shivaprasad, 2000; Hong et al., 2013).

S. pullorum is the pathogen that causes the pullorum disease in poultry. This pathogen is responsible for targeting the bursa of Fabricius while causing intestinal inflammation (Qiuchun et al., 2013). *S. pullorum* persists in spleen macrophages which will cause infections in the reproductive tract and consequently cause vertical transmission causing infections to eggs and offspring (Henderson et al., 1999; Wigley et al., 2001).

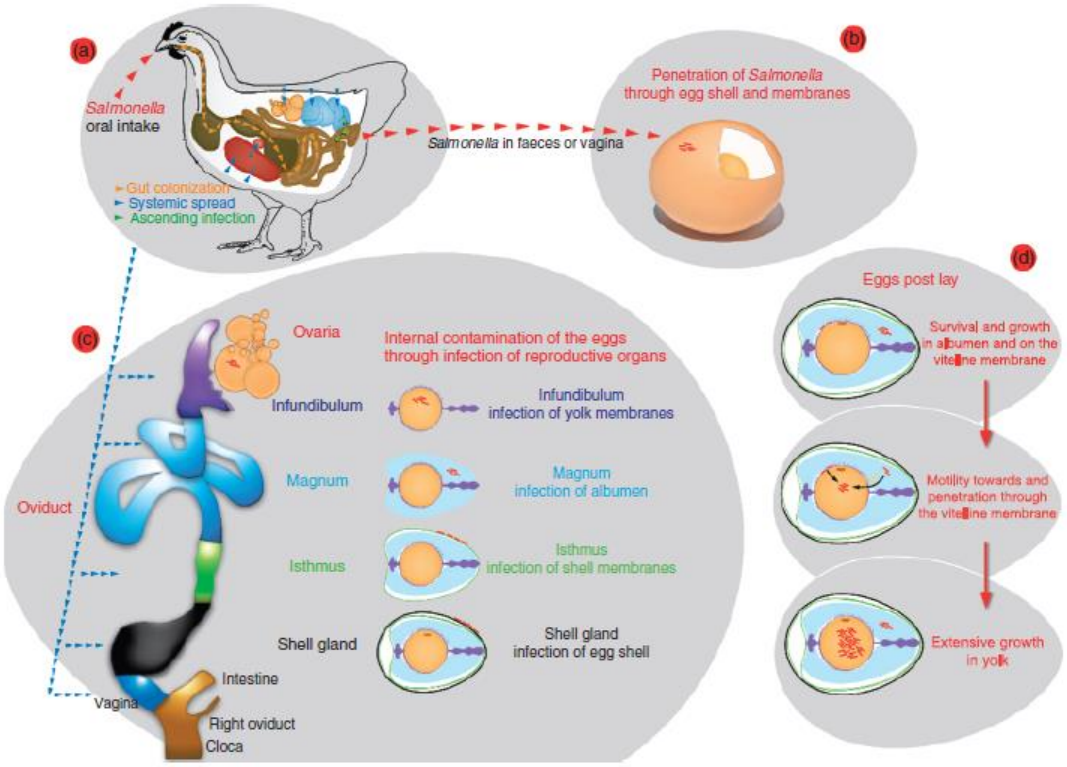

Figure. 1. Pathogenesis of egg contamination by *Salmonella*. (Gantois et al., 2009).

Salmonella enterica serovar typhimurium (*S. typhimurium*) is a pathogen that can modify the signaling pathways that conform the several barriers of the mucosal epithelium. This mucosal epithelium in poultry is designed to prevent the adhesion of pathogenic bacteria, *S. typhimurium* modifies the effector proteins in the epithelial cells causing bacterial entry and inflammation (Patel and McCormick, 2014).

Eggs can also be contaminated with *Salmonella* by two routes among many others. One of the routes that an egg can be contaminated is by the penetration of the eggshell within a colonized gastrointestinal tract. Another possible way for an egg to be contaminated is by direct contamination of any of the egg membranes and/ or yolk before oviposition, this can occur from reproductive organs that are infected with *Salmonella* (Gantois et al., 2009). *Salmonella* that remain in the albumen or vitelline membrane are able to survive antimicrobial environment, afterwards they migrate to the yolk and begin to multiply (Figure 1; Gantois et al., 2009).

Salmonella enters the intestinal tract of poultry through orally means. Bacteria begin colonizing the intestinal lumen and attach themselves to competitive binding sites. Macrophages go to the invasive site and enclose the *Salmonella* bacteria, permitting the *Salmonella* pathogens to survive and multiply within the macrophage. These infected macrophages migrate to the reproductive organs. *Salmonella* can also infect the cloaca and migrate to the oviduct.

According to the Department of Health of Puerto Rico, in 2005, 692 cases of Salmonellosis were registered, however, these were not of foodborne origin. The Department of Health of Puerto Rico also reported that in 2006, 774 cases of Salmonellosis, and 18 cases of Campylobacteriosis were reported and these were of foodborne origin (Departamento de Salud de Puerto Rico., 2006).

3.2 *Campylobacter*

The genus *Campylobacter* is a macroaerophilic, spiral-shaped, motile Gram-negative bacilli with unipolar or bipolar flagella (Ewing et al., 2007). It is relatively fragile, and sensitive to environmental stresses. In recent years, Campylobacteriosis has been emerging as a major bacterial food borne disease in industrialized countries (Epps et al., 2013). Mainly *Campylobacter jejuni*

(*C. jejuni*) is associated with acute *Campylobacter enteritis* in humans causing more than 80% of the registered Campylobacter infections (Hofreuter, 2014). In the United States, the annual incidence of infection with *C. jejuni*, which causes Campylobacteriosis is estimated between 2.4 to 4 million cases (Neal-McKinney et al., 2014).

The clinical signs for Campylobacteriosis are not distinct from Salmonellosis, and range from mild watery to severe, inflammatory and bloody diarrhea along with abdominal pain and fever (Allos, 2001). The symptoms have large variations, most commonly it begins with muscle pain, headaches and high fever (Lawley, 2013). Nausea can occur, however vomiting is rare, diarrhea is also common and the presence of blood and mucus in the feces can also occur. On occasions, Campylobacteriosis can have serious complications, such as arthritis in the form of Reiter's syndrome (Lawley, 2013).

C. jejuni and *C. coli* are recognized among most of the various species of *Campylobacter spp.* by their high optimum growth temperature at 42° C. From this information, one can infer that Campylobacter does not multiply during slaughter, post processing, transport and refrigeration of chicken products. Even if it does not multiply, Campylobacter still persists for prolonged periods in chilled frozen products, a reduction in the concentration and viability has been recorded in several weeks of storage at 4°C (Food and Agriculture Organization of the United Nations, 2009).

Incidents of carcass contamination of broilers with *C. jejuni* has increased in the recent years (Hopkins and Scott, 1983). Chicken livers and wings are among some of the most well-known parts of the carcass that have been contaminated (Barot et al., 1983; Kinde et al., 1983). The transmissions of poultry borne infection diseases like Salmonella and Campylobacter are a public health concern (Balsley, 2006; Cardinale et al., 2003) as chicken meat is largely consumed by humans. Human campylobacteriosis has transformed into the most common cause of food

poisoning in the poultry industry (Tauxe, 2001), and the only known method of managing this disease is to either by reduction and/or elimination of *C. jejuni* in the food chain. Most infections of *C. jejuni* are caused by chicken farms and processing plants, as chickens are the major reservoir of *C. jejuni* (Solomon and Hoover, 1999; Ahmed et al., 2002).

In order to assess this public health problem many methods have been implemented to reduce the incidence of infected carcasses. One of the characteristics that affects finding a method to reduce the incidence, lies within the pathogen itself, as Campylobacter can heavily colonize the broilers intestinal mucosa and still have the capability to resist and evade the broilers immune responses (Achen et al., 1998). The dissemination of *C. jejuni* occurs through fecal shedding, this has been evidenced as *C. jejuni* has been found in the feces of infected broilers. As one member of the flock has been contaminated, the transmission of *C. jejuni* throughout the rest of the flock occurs rapidly. C. jejuni develops its colonization in the mucus of the epithelial cells in the ceca and the small intestine of broilers (Cawthraw et al., 1994). The colonization of *C. jejuni* persists during the entire life span of the broiler, which is usually 47 days in commercial broilers.

The incidence of human campylobacteriosis is increasing to the point that it has become the most common cause of food poisoning (Tauxe, 2001). To prevent this, vaccinations have been implemented, however these had only partial effectiveness on *C. jejuni* colonization in Broilers (Khoury and Meinersmann, 1995). The optimal antigen or antigens necessary to create an effective vaccine against *C. jejuni*, is a challenge since many aspects of C. jejuni are still under investigations including pathogenesis and metabolism.

3.3 Prebiotics and Probiotics

The health and nutritional status of poultry is largely connected to the gastrointestinal microflora, in which it can directly or indirectly have effects on the gut morphology, nutrition, the pathogenesis of intestinal diseases and the immune system response (Lan et al., 2005). The gastrointestinal tract of poultry comes in contact with exogenous microorganisms immediately after hatching and afterwards it becomes an environment which supports the development of complex microbiomes that consist primarily of anaerobic bacteria (Pan and Yu, 2014). Poultry such as chickens, turkeys and ducks, have shorter gastrointestinal tracts and faster digestive transit (Pan and Yu, 2014).

Food-borne illnesses are a public health concern, and the increased incidents of human campylobacteriosis and salmonellosis are causing poultry farms and different organizations including USDA, to increase security health measures. The widespread use of antibiotics as a feed additive to prevent illnesses for cattle, porcine, and poultry has created resistance in many strains and species of pathogens. This resistance has caused the use antibiotics in feed additives as growth promoters to be forbidden in many parts of Europe (Hou et al., 2015).

As additives, antibiotic growth promoters are used in quantities that cannot treat diseases in the broiler. Such subtherapeutic quantities can greatly improve the growth rates and feed efficiency of poultry (Dibner and Richards, 2005), with the risk of developing resistance in some bacterial populations. As result of preventing future resistance in pathogens, alternatives are being pursued. Alternatives such as prebiotics and probiotics are among the options that have been considered to prevent food borne illnesses.

Prebiotics are non-digestible additives in feed that are generally polysaccharides and oligosaccharides. These beneficial bacteria in adequate amounts can limit the proliferation of

pathogens such as Salmonella and Campylobacter by promoting the growth of lactic acid bacteria in the colon of broilers.

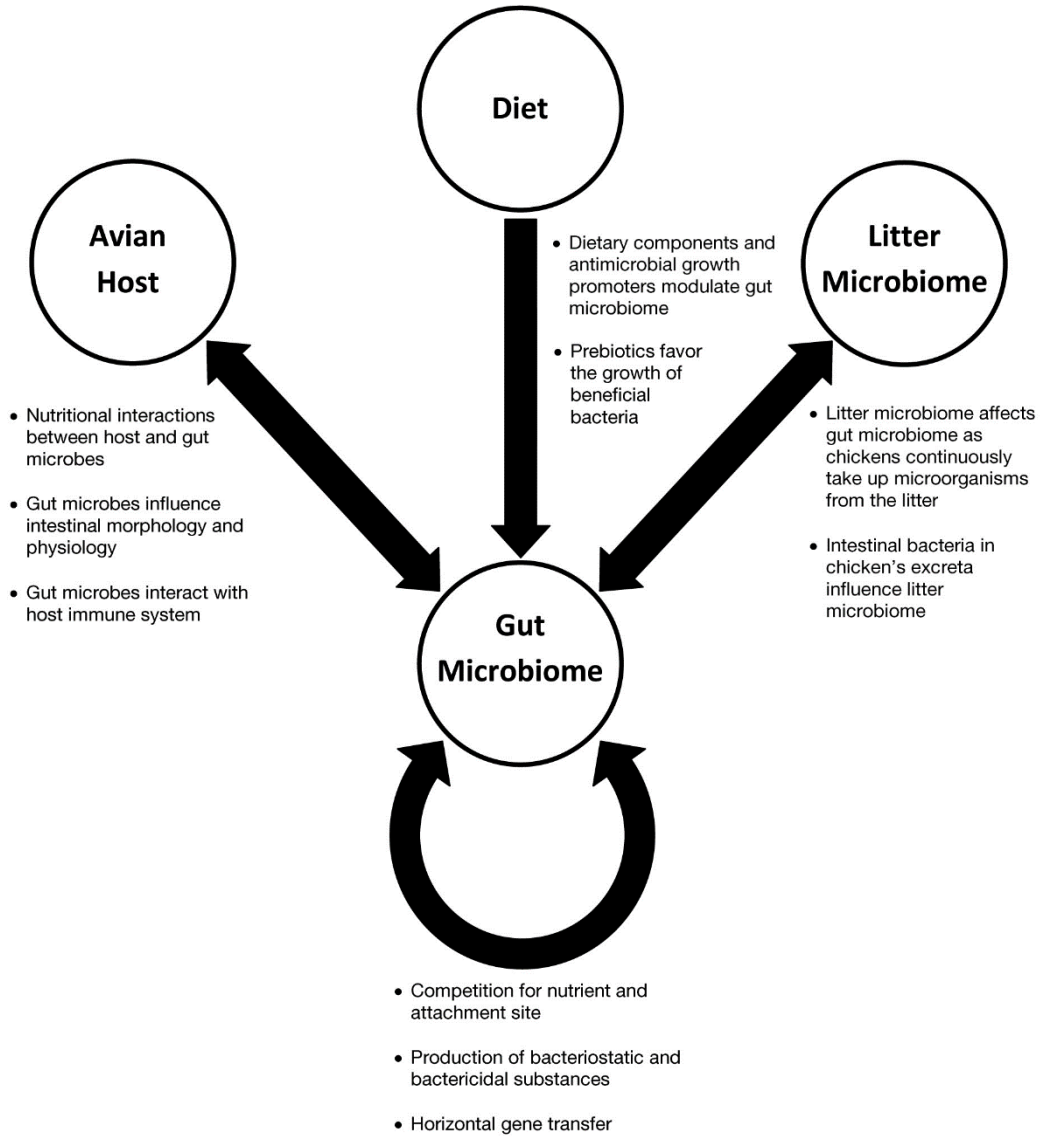

Figure 2. Conceptual model of the interactions among gut microbiome, avian host, diet, and litter microbiome (Pan and Yu, 2014).

Prebiotics adhere to competitive binding site in the intestinal mucosa of the broiler ceca, by this method, pathogens such as Salmonella and Campylobacter cannot adhere and cause the broiler harm. The beneficial effects of prebiotics consist of using complex carbohydrates for beneficial

bacteria nutrition which creates a beneficial environment in the prevention of pathogens (Nava et al., 2005).

Prebiotics such as *Mannan-oligosaccharide* (MOS) and *Fructo-oligosaccharide* (FOS) are among the most tested prebiotics in the poultry industry (Janardhana et al., 2009). Trials done on broilers using MOS as a feed additive have shown an improved FCR, BWG and reduced mortality (Hooge, 2004). FOS has demonstrated to improve the feed efficiency and decrease the incidence of diseases and mortality (Chen et al., 2007; Bornet et al., 2002; Fukata et al., 1999).

In a trial performed by Kim et al. (2011), FOS and MOS were used to study the performance of 240 Ross broilers. The objective of this trial was to study the effects of FOS and MOS on different concentrations in the diet of broilers. The trial did not demonstrate any significant results between control and the supplemented broilers in terms of mortality, FI and FCR (Kim et al., 2011). Real-time PCR demonstrated that broilers fed with FOS and MOS caused a reduction in pathogens ($P<0.05$) in the small intestine such as *Clostridium perfringens* and *E. coli*. These changes occurred for an inclusion rate in the diet for FOS at 0.25% and an inclusion rate for MOS at 0.05%. The results obtained in this study, demonstrate that for the use of the additives in diets, an appropriate inclusion rate must be utilized, so that the effects on the performance of the broiler can be observed.

Probiotics are non-digestible living microorganism that are utilized as feed additives on poultry, porcine and cattle to improve the immune system. These are an alternative to antibiotics since these beneficial bacteria can also improve the efficiency of feed utilization and improve the gastrointestinal ecosystem (Hou et al., 2015). As probiotics operate in adhere to competitive binding sites preventing the adhesion of pathogens. This type of microbial modification can also

benefit the intestinal epithelium environment, improving conditions that benefit the beneficial bacteria, promoting an improved FCR (Rolfe, 2000).

Lactobacillus and *Saccharomyces cerevisiae*, have been studied and tested in poultry. Trials with both of these bacteria, in adequate amounts, are able to improve carcass characteristics (Weis, 2011) and meat quality (Endo, 1990).

Positive effects of numerous probiotics organisms such as: *Lactobacillus* and *Saccaharomyces cerevisiae*, have been reported in chickens (Vali et al., 2013), dietary supplementation of broiler diets with probiotics have been demonstrated to improve carcass characteristics (Weis, 2011) and meat quality (Endo and Nakano, 1990). *Lactobacilli* are one of the predominant bacteria that possess properties of acid and bile salt tolerance and also possesses superior capability for colonization and adhesion, in the gastrointestinal tract of poultry (Wang et al., 2014). These characteristics make *Lactobacilli* a suitable candidate to endure the many adverse effects of the gastrointestinal tract of poultry, such as pH.

The various species of Bacillus subtilis (BAC) possess bacteriocin-like inhibitory substances, these substances have a role as auto inducers in the activation of gene clusters. BAC also exhibits a broad spectrum of antimicrobial activity, additionally it remains stable in a wide-range of temperatures and pH. As bacteriocins BAC has potential to prevent or control both spoilage and pathogenic microorganisms. BAC is mostly applied as a probiotic for human use, as animal feed and are found in the food supply as preservative (Mongkolthanaruk, 2012).

Aliakbarpour et al., (2012) showed that the inclusion of Bacillus subtilis as a probiotic in diets to had no significant effect ($p>0.05$) in terms of total FCR, while comparing control with BAC supplemented broilers. Significant differences ($P<0.05$) were found in the growth performance,

and final BW when compared to the control group ($p<0.05$). Additional research is required to assess the results of this investigation, since other similar trial have been performed resulting in no effects on the broilers. Probiotics are feed additives that are only effective to the host when used in specific quantitative pattern, and different species of probiotics are taken into account to compare to the hosts intestinal morphology (Aliakbarpour et al., 2012).

EFFECT OF A DIETARY PREBIOTIC AND PROBIOTIC ON BROILER PERFORMANCE AND CARCASS YIELD

4 MATERIALS AND METHODS

4.1 Broiler Management

There were 400 Cobb chicks that were hatched at the hatchery facilities of To-Ricos, Aibonito, Puerto Rico. The chicks were transported to a livestock research facility in Lajas, Puerto Rico, were they were raised in floor pens. This research house had 20 pens, where 30 randomly selected broilers were placed regardless of gender. Each pen had nipple drinkers, a tube feeders and brooder lamps. Each pen had also fresh coffee husk as litter material. Natural light entered both sides of the house through open or translucent curtains during day light hours. Temperatures of the house was natural, and no temperature control was utilized. All performance factors were determined for each pen, regardless of gender. Feed and water were verified and refilled for the entirety of the 41 d of the study. Mortality was verified every day for the 41 d of the study and carcasses were removed from the pens and disposed appropriately.

4.2 Broiler Diets and Dietary Treatments

During the rearing period, each broiler pen received a Broiler starter – 1lbs/ bird at 7 days of age. These were fed in a feeding pan on the pen floor, and feeding was *ad-libitum*. Starting at 14 d of age, each Broiler pen was assigned randomly one of the 4 dietary treatments. These treatments were Control (CON) – Commercial Control (CON), MOS – Control + (0.1% inclusion rate), BAC – Control + (0.025% inclusion rate), M + B – Control + MOS + BAC. The design was randomized complete block with 2 blocks (house rows – East and West Ends of House 1). Each of these inclusion rates and additives were also added to the diets for the 28, 35 and 41 d of age. All dietary

stages were provided by To-Rico (starter, grower, WD#1 and WD#2). All pens had full water access in nipple drinkers.

Table I. Calculated analysis for broiler dietary treatments for the Starter, Grower, Withdrawal #1 (WD#1), and Withdrawal #2 (WD#2).

Calculated Nutrients, %	**Diet**			
	Starter	**Grower**	**WD #1**	**WD #2**
Crude Protein, min.	19.0	18.0	15.0	14.75
Lysine, min.	1.18	1.05	0.95	0.80
Methionine, min.	0.54	0.50	0.45	0.38
Crude Fat, min.	3.00	3.00	3.00	3.00
Crude Fiber min.	4.00	4.00	4.00	4.00
Calcium, min.	0.65	0.60	0.55	0.50
Calcium, max.	1.00	0.90	0.85	0.85
Phosphorus, min.	0.40	0.45	0.38	0.35
Salt, min.	0.39	0.39	0.39	0.39
Salt, max.	0.62	0.62	0.62	0.62
Sodium, min.	0.15	0.15	0.15	0.15
Sodium, max.	0.24	0.24	0.24	0.24
Phytase (Pichia pastoris), FYT/lb	250	250	250	250
NSP*	1.0	1.0	1.0	1.0
Salinomicine, g/ton	-	60.0	50.0	-
Disalate of bacitracin of methylene, g/ton	-	50.0	-	-

The exact quantity information of each of the dietary ingredient is proprietary of Pilgrims Inc. and is not available for publication. Dietary ingredients: Grain Products, Plant Protein Products, Animal Protein Products, Animal Fat and Hydrolyzed Vegetable Oil (preserved with Ethoxyquin), Calcium Carbonate, Monocalcium Phospahate, Salt, Methionine Supplement, L-Lysine, L-Threonine, Vitamin A Supplement, Cholecalciferol (source of Vitamin D3), Vitamin E Supplement, Menadine Sodium Bisulfate Complex (source of Vitamin K), Vitamin B-12 Supplement, Riboflavin Supplement, Calcium Pantothenate, Betaine, Niacin Supplement, Thiamine Mononitrate, PyridoxineHydrochloride, Folic Acid, Biotin, Copper Sulfate, Ethoxyquin (a preservative), and Traces of Manganous Sulfate, Copper Sulfate, Ferrous Sulfate, Calcium Iodate and Sodium Selenite. Sodium Sulfate, Kaoline, Cellulose, Hydrogenated Vegetable Oil, Dextrin, Starch, Condensed Fermented Corn Extractive, Dehydrated Pichia pastoris Fermentation Extract and Trichoderma Longibrachiatum Fermentation. These products are free of antimicrobial activity and are not a source of viable microbial cells. *Non-Starch Polysaccharide Enzyme - NSP

4.3 Data collection:

At 7 days of age the chicks were offered 0.5 pounds of crumbled broiler starter per bird. Pen BW and weigh backs were recorded during this period. At 14, 35 and 40 days of age pen body and weigh backs were recorded. All feeds were adjusted per bird alive prior to adding the next stage of feed. At 40 days of age 3 birds per pen were randomly selected, slaughtered, and the ceca was removed to determine the presence of *Salmonella spp.* and *Campylobacter spp*. Mortality was taken into account for all pens, and each carcass was disposed of appropriately. Each pen had its BW determined according to the broilers that were alive at the moment of weighting.

4.4 Qualitative RT - PCR for the detection of *Salmonella spp.* and *Campylobacter spp.* in poultry ceca content

To verify the absence or presence of *Salmonella spp.* the *mericon Salmonella spp.* kit was utilized. This kit is designed by QUIAGEN®. Following the instructions by the manufacturer, the protocol for the detection of pathogen DNA by real time PCR without ROX was utilized for 96-sample kit. This kit utilized 1040 µl of Multiplex PCR Master Mix mixed with a vial of *mericon* Assay, this mixture creates a Reconstituted *mericon* Assay. A quantity of 5µl of the Reconstituted *mericon* Assay, along with a sample of 5µl of the DNA obtained from the contents of the randomly chosen Broilers from each pen, will be placed in a RT - PCR. Each of samples obtained went through the process of extraction of DNA, as instructed by the manufacturer, which was a cycling protocol for real-time cyclers (QIAGEN, 2012). The results obtained in the RT-PCR were compared to the control reactions for Positive and Negative PCR results. The results would only determine the presence and absence of Salmonella spp. for the 3 samples obtained from each pen.

This same procedure was used for the detection of *Campylobacter* except the *mericon Campylobacter* kit was utilized. This kit was designed by QUIAGEN® for the qualitative detection of Campylobacter subspecies. The rest of the procedures for the extraction of DNA from the samples and the RT-PCR run, were the same as for *Salmonella spp.*

4.5 Statistical analysis

Statistical analysis was performed using the software InfoStat. While utilizing InfoStat the data is presented in means using ANOVA. All of the results for all variables: FI, CFI, BW, BWG, CFCR, AdjCFCR, and Mortality were represented as means and SEM, in which a $P<0.05$ and $P<0.01$ was considered significant. For the analysis of RT – PCR a chi squared test of independence was performed in which the number of positive and negative detections were evaluated as a frequency. A chi squared p – value ($P<0.05$) was considered significant.

5 RESULTS AND DISCUSSION

In order to ascertain the effectiveness of MOS and BAC as additives in poultry, variables such as FI, CFI, BW, BWG, CFCR, and AdjCFCR were determined to assess poultry performance (Table II).

It was observed that M + B birds consumed more feed when compared to CON and MOS ($p<0.05$, Table 1) for the period 1 – 14 d, while BAC remained as an intermediate. During the period of 14 – 28 d, MOS, BAC and M + B has a higher FI when compared to CON ($p<0.05$). During the period of 28 – 35, CON tended to consume more feed when compared to MOS, while BAC and M + B, demonstrated a statistical trend ($P = 0.10$). From 35 – 40 d of age, CON had the highest FI, compared to its counterparts ($P < 0.01$, Table I).

In a trial performed by Giang et al., (2011), two probiotics were used for dietary supplementation and observe performance parameters in growing-finishing pigs. Using *Bacillus* and *Saccharomyces* in their diets, the results of this trial demonstrated that supplemented diets with probiotics had no effect on FI, ADG and FCR ($p>0.05$) when compared to the control group. Ordinarily, since the microbial flora of the pig is unstable during the first weeks of post-weaning, a supplementation of probiotics in the diet of pigs will be able, theoretically, to increase immunological defenses until the pig's gastrointestinal tract is stabilized (Jensen, 1998; Giang et al., 2011). This is what is supposed to occur. However, studies have shown contradictory results. During the experimental period, the pigs were fed ad libitum and to measure the growth performance the factors of daily FI, ADG and FCR were taken into account. Both of the probiotics inclusion diets were not effective in improving average FI ($p>0.05$) when compared to the control group. FCR and ADG were improved ($p<0.05$) compared to the control group. The finisher period group of pigs demonstrated no effect on any of the performance parameters that were taken into

account in any of the probiotic treatments. The reason for these responses is that probiotics such as *Bacillus* and *Saccharomyces* are foreign microorganisms that do not naturally colonize the pig's gastrointestinal tract (Chesson, 1994; Kornegay and Risley, 1996). Both probiotics are able to prevent the colonization of multiple pathogens in the periods of grower and finisher pigs. Since the gastrointestinal tract is already stable, the effects of both probiotics is reduced to the point that it causes no effect on the performance, when compared to its effects during the post-weaning period (Jensen, 1998; Nousiainen and Setälä, 1998). Other studies have found contradictory results, however, many of these trials use different strains of probiotics along with different basal diets. Inclusion levels, specific strains of probiotics and prebiotics, a mixture of strains, and many other factors can cause the performance parameters to increase. However, since a standardized use of these additives is not established, many trials find themselves observing from small improvements to no effects in performance parameters in pigs and other animals as well (Davis et al., 2008; Bowman and Veum, 1973; Wang et al., 2009).

Table II. Effect of dietary treatment on broiler's feed intake (FI), cumulative feed intake (CFI) Body Weight (BW), Body Weight Gain (BWG), Cumulative Feed Conversion Ratio (CFCR), Adjusted Feed Conversion Ratio (AdjCFCR), and Mortality.

Variable	Age, d	Dietary Treatment				SEM	p-value
		CON	MOS	BAC	M + B		
FI, lb	1 – 14	0.93b	0.91b	0.94ab	0.98a	0.01	<0.05
	14 – 28	2.41b	2.60a	2.62a	2.56a	0.04	<0.05
	28 – 35	2.32x	2.07y	2.23xy	2.18xy	0.07	0.10
	35 – 40	2.02A	1.72B	1.85B	1.77B	0.04	<0.01
CFI, lb	1 – 14	0.93b	0.91b	0.94ab	0.98a	0.01	<0.05
	1 – 28	3.34b	3.52a	3.56a	3.53a	0.05	<0.05
	1 – 35	5.66	5.59	5.80	5.71	0.08	NS
	1 – 40	7.68a	7.31b	7.65a	7.49ab	0.10	0.05
BW, lb	14	0.84	0.86	0.86	0.88	0.01	NS
	28	2.88	2.92	2.90	2.81	0.05	NS
	35	4.08	4.12	4.16	4.06	0.06	NS
	40	4.96	4.97	5.11	4.87	0.08	NS
BWG, lb	1 – 14	0.75	0.76	0.77	0.78	0.01	NS
	14 – 28	2.03	2.05	2.00	1.93	0.04	NS
	28 – 35	1.15	1.20	1.27	1.22	0.05	NS
	35 – 40	0.89	0.84	0.88	0.82	0.03	NS
CFCR, lb:lb	1 – 14	1.25	1.20	1.23	1.26	0.02	NS
	1 – 28	1.32B	1.37A	1.40A	1.42A	0.02	<0.01
	1 – 35	1.69	1.61	1.63	1.67	0.03	NS
	1 – 40	1.96	1.83	1.83	1.90	0.05	NS
AdjCFCR, lb:lb	1 – 14	1.23	1.20	1.23	1.24	0.02	NS
	1 – 28	1.31A	1.37B	1.39B	1.41B	0.01	<0.01
	1 – 35	1.68	1.60	1.63	1.66	0.03	NS
	1 – 40	1.94	1.83	1.82	1.90	0.04	NS
Mortality, %	1 – 14	4.00	1.34	1.42	5.28	1.79	NS
	1 – 28	4.68	2.66	2.80	7.28	1.93	NS
	1 – 35	5.34	2.66	2.80	7.28	1.86	NS
	1 – 40	5.34	2.66	2.80	7.94	1.95	NS

AB Means across columns lacking a common superscript are significantly different at the $P < 0.01$.
ab Means across columns lacking a common superscript are significantly different at the $P < 0.05$.
xy Means across columns lacking a common superscript are significantly different at the $P < 0.10$.
The four dietary treatments consisted of CON = Commercial Control (CON), MOS = Control + MOS (0.1% rate of inclusion), BAC = Control + BAC (0.05% rate of inclusion), and M + B = Control + MOS + BAC.

A trial was performed by Silva et al., (2010), the inclusion of prebiotics and yeast extract was used to evaluate the performance of broilers under different temperatures in the broiler's pre-initial phase. During the pre-initial phase more than one thousand chicks were offered the yeast extract and prebiotic. In an experimental design in which the environmental temperatures were hot, thermoneutral or cold, each of these chicks were fed with each respective diet be that with or without yeast extract and with or without the prebiotic. The performance of the chicks was evaluated at 42 d of age, for FI, FCR, BW and viability. The hot temperatures with the inclusion of prebiotics causes a villus height in the duodenum. Villus height are small projections in the epithelium that aligned and conform the epithelium mucosa. As the temperature rises the villus height elevates and that increases a higher efficacy in the feed absorption. In cold temperatures with the inclusion of prebiotics occurs an increased villus height in the duodenum and jejunum. An increased weight gain in the cold temperature causes elevated levels in FI, which in this case the broilers will use it to raise their body temperature (Close and Mount, 1978). In the hotter environments the decreased levels of FI occur to reduce the heat levels arising from the bird's metabolism. The results of this experiment showed an increased BW and FI in cold temperatures compared to its counterparts. However, to the birds that were not treated with prebiotics, the villus height was lower and FCR was less efficient compared to its counterparts.

The cumulative FI in the present trial, in the period of 1 – 14 d, was greater for M + B birds when compared to CON and MOS ($P<0.05$, Table I). In the period of 1 – 28 d CON ($P<0.05$, Table I), the also consumed less feed when compared to the other treatments. CON demonstrated a higher cumulative feed intake ($P<0.05$) when compared to the treatment MOS, in the period of 1 – 40 d.

All dietary treatments demonstrated no statistical significance in BW and BWG. These results are in agreement with investigations performed by Midilli et al., 2008, Alp et al., 1993 and Waldroup

et al., 2003. These trials demonstrated the lack of effect of probiotic or prebiotic supplementation in relation to BW, BWG, FI, and FCR. However, Piray et al., (2007), performed a trial in which the use of Fermacto® (aspergillus mycelium meal) was added in the broilers feed as a prebiotic. This trial resulted in a significant increase in BWG and FCR to ($p<0.05$) on broilers fed with Fermacto® from 14 d-of-age and older. However, this result was not observed on the first and second week of life.

In a study by Chen et al., (2005), two different strains of probiotics were used to evaluate the effects on the growth performance on growing pigs. Ninety pigs were used in this trial to observe the effects of *Saccharomyces cerevisiae* and *Bacillus subtilis* in three dietary treatments. The variables assessed were BW, ADG, FI, among other variables. This trial demonstrated that a significant increase ($p<0.05$) in ADG when pigs were fed in 0.2% probiotic inclusion, when compared to 0.1% of inclusion. However, FI and gain/feed were not affected by the probiotic dietary treatment. There are contradictions to this result as, Pollman et al., (1980) did not find any effects on growth performance on probiotic supplemented diets, suggesting that significant effects will be noticeable during an earlier stage in the pig's life. Lessard and Brisson (1987), encountered a higher BW with nursery pigs that were also supplemented with probiotics in their diets. All of these different results are theoretically risen due to many factors, such as type of strain of bacteria, inclusion rate in the diet, feeding schedule and diet composition (Chen et al., 2005). All of these factors come together to create an effect that may or may not present itself in the pig's performance or in this case, the broiler's performance. This is another reason for the many inconsistencies within the different species of animals that undergo this type of treatment.

A trial performed by Shahir et al., (2014), used a specific probiotic (Biosof®) and a prebiotic (Active-Mos®) to observe broiler performance, and other health parameters to prevent illnesses

such as influenza and Newcastle. The three hundred twelve 7 – d old male broiler chicks had an improved BW and FCR in dietary inclusions of prebiotic and probiotic ($p<0.05$) when compared to the control diet (Shahir et al., 2014). An interesting observation among the broilers that were fed probiotic and prebiotic, is the fact that the relative weight of the duodenum and the jejunum was significantly higher compared to the control group. This increase in relative weight of the small intestine is associated to a morphological and histological changes, an increased absorption of the surface of the epithelium, and a lower count in pathogenic bacteria (Awad et al., 2009). Other observations that were discovered with broilers that were fed with probiotics and prebiotics was that the total protein, albumin, globulin, and albumin to globulin ration, was not affected in any way. These observations during the trial demonstrated that the probiotic diets had a cholesterol decreasing effect on the birds (Shahir et al., 2014). The reason for this cholesterol decrease is believed to be associated to the de conjugating of bile salts that are caused by the probiotic bacteria. These bacteria cause that the cholesterol is absorbed in lesser quantities and excreted in higher quantities (Klaver and Meer, 1993).

Another trial that was performed by Kanakupt et al. (2011), demonstrated the use of prebiotics such as FOS and *galactooligosaccharides* (GOS) in adult cats. The trial was performed to analyze the effects of prebiotics in the cat's nutrient digestibility, fermentative end product production and fecal microbial ecology. Dietary supplementation with each treatment was FOS and GOS individually, FOS + GOS and the control group, and each treatment had an inclusion rate of 0.5% and 1%. Results of this trial showed a decrease in crude protein (CP) digestibility ($p<0.05$), for a GOS + FOS diet. Each of the cats that were treated with dietary supplements (FOS, GOS, and FOS + GOS), demonstrated an increase in the *Bifidobacterium spp.* colonies ($p<0.05$) compared to the cats of the control group. The pH in the fecal matter of cats fed with FOS + GOS, was less ($p<0.05$)

than those compared with cats in the control group. The observation of a lesser CP digestibility began to decrease in the FOS + GOS inclusion of 1% supplementation. The reason for this decrease lies in the increase in the bacterial biomass of the large intestine. This kind of decrease reveals that the effects occurred because of an increased oligosaccharide concentration and not because of an individual species of oligosaccharide that was included (Kanakupt et al., 2011). The decreased pH in fecal matter was another observation that was seen in cats that were fed FOS + GOS when compared to its counterparts. The low pH in fecal matter is believed to happen due to an increased production of lactic acid and short-chain fatty acids that occur from the carbohydrate fermentation (Kanakupt et al., 2011).

The CFCR when adjusted for mortality (Table II) from the period of 1 – 28 d, demonstrated that the treatments for MOS, BAC, and M + B had less efficiency when compared to CON ($p<0.01$). Theories have stipulated that probiotics have the property to improve efficiency by altering the intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and Gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and the enhancement of digestion and utilization of nutrients (Yeo and Kim, 1997). However, no effect on FCR were observed in this trial, which also agreed with the findings by Willis and Reid, 2008. However, this trial found significant differences ($p<0.05$) in feeding regimens among its groups in the days 21 to 42 (Willis and Reid, 2008, Amerah et al, 2007). This trial was performed to verify the presence of *C. jejuni*, using a specific probiotic while offering a strict diet regimen (ad-libitum, skip-a-day and 8hrs restricted diet) to five hundred forty broilers, that were also divided in 15 males and 15 females in each pen for a duration of 49 d. In general, the dietary restricted regimen in 21 d, and the 49 d on the *ad-libitum* and skip-a-day diet regiment, demonstrated significant differences when compared to the control and experimental group

concerning the feed type. BW was taken into account on gender differences as well. For males the administration of feed for *ad – libitum* and 8 hrs. restricted diets was higher when compared to the skip-a-day regiment. For females the BW was significantly different between each of the regimens, with *ad-libitum* being the highest and skip-a-day being the lowest. When both genders were analyzed for BW with probiotic inclusion, the BW was different among the regimens ad-libitum being the highest and skip-a-day being the lowest. The trial supports the idea that the inclusion of probiotics depends on factors like stress, diet, environment, feed administration, the age of the bird and even gender (Ewing and Cole, 1994; Patterson and Burkholder, 2003). Probiotic inclusion in the diets of broilers can vary in effect due to many factors, such as gender and the feeding schedule.

Many trials have demonstrated that probiotics and prebiotics improve in the feed conversion of broilers, however the information is lacking on the physiological, immunological, and in vivo microbiological modes of action. Poultry producers find themselves often with many questions on which specimen and how much of the dietary supplementation or even if they should make inclusions in their flock's diet.

The factor that was previously discussed in this trial are by definition the FCR. FCR is affected by the BW, FI among other traits, which ultimately determines the broilers' performance. For an improved feed efficiency on a biological level, certain ways can be implemented. According to the energetic model and trial developed by Teeter and Skinner-Noble, 2003, efficiency can be improved by reducing the days necessary for the broilers to reach market weight. According to this trial nearly half of the maintenance energies are attributed to many factors such as bird activity, alterations of the use of metabolized energies to meet maintenance as whole. If a reduction in maintenance energy is accomplished, then a decrease in basal metabolic energy on the broilers would be in effect.

If a reduction in the requirements of metabolized energy for maintenance is partly responsible for an improved feed conversion, the same effects can be attributed to the trial that was performed. However, in the trial that was performed, the FCR improvement was only numerical.

Prebiotics can have the capacity to alter the intestinal microbes and immune system to reduce the colonization by the pathogens in certain conditions (Hajati and Rezaei, 2010). The two most commonly used oligosaccharide with prebiotic characteristics are *fructo-oligosaccharide* and *mannan oligosaccharide*. *Mannan-oligosaccharide* was the prebiotic used in the trial that was performed. This prebiotic found in the cell wall of yeast, has mannose as its main component. Mannose is bound by the type I fimbriae which is used by many species of enteric bacteria in order to attach itself to the host cell. As an additive mannose can have the effect of the movement of pathogenic bacteria through the intestine without colonization (Newman, 1994).

The inclusion of MOS in broiler diets confers many effects, among them is the increased production of Immunoglobulin A. This Immunoglobulin A (IgA) will act on the intestinal epithelium by competitive attachment, while it attaches to binding sites, this prevents the adhesion of pathogenic bacteria avoiding illnesses as well as inflammation that could cause further damage to the epithelial tissue (Russell et al., 1989).

The supplementation of BAC as an additive behaves like any other probiotic, they work through competitive adhesion to prevent the colonization of pathogenic bacteria. However, BAC has been known to create a favorable anaerobic environment for the proliferation of lactobacilli that is native to the broiler's GI tract (Jeong and Kim, 2014).

The CFCR was significantly better for CON from 1 – 28 d-of-age. If the supplementation of MOS causes an increase in the production of Immunoglobulin A, this will cause an increase in the use

of energy directed at the immune system in order to prevent the attachment of pathogens. This redirection of energy to the immune system causes too much stress on the body of the broiler, which would explain why the broilers that were fed additives did not demonstrate a significant difference ($p<0.05$) in feed conversion. The reason behind the fact that no significant differences were found in the days of 1 – 35 and 1 – 40, is because much like direct-fed antibiotics, probiotics are not completely effective in all types of illnesses or events throughout the bird's life. The effectiveness of both relies on the in a more ill ridden environment, in which the nutrients that are absorbed through feed are used to increase support to the immune system. This event occurs because there is an increment in the pathogenic bacteria and increased production of cytokines that enable inflammation (Klasing and Johnstone, 1991). Other trials have also dealt with phytogenic blends; these are blends derived from plants that have antibiotic properties that can be used as additives in feed. While some trials have observed an increased activity in pancreatic trypsin and amylase, the broilers' performance was not improved in terms of BW and FCR (Jang et al., 2007, Jang et al., 2004). A statistical difference in the present trial could emerge if a higher number of replications were performed. Since the present study had a small number of replications, results in the present study cannot demonstrate the full extent of the effects the dietary treatments on the birds.

Table III demonstrates the effects of dietary treatment on the broiler on the live body weight, carcass weight and carcass yield. Among all the treatments during the 1 – 40 d, no statistical significant differences were found for mortality, carcass weight and carcass yield (Table III). In a trial performed by Pelicano et al., (2003), different probiotics were used in one thousand and fifty Cobb male to ascertain its effects on the broiler's carcass and meat quality. The different probiotics studied were two strains of *Bacillus, Saccharomyces cerevisiae and two strains of Lactobacillus*.

The Control group demonstrated a superior carcass yield ($p<0.05$) when compared to the groups that were treated with probiotics. Among the observations of the groups that were treated with probiotics, was a decrease in color and pH in the muscle breast after slaughter. However, the group that showed a higher leg yield ($p<0.01$) were broilers that were fed probiotics. Probiotics fed groups also demonstrated numerical reduction in abdominal fat.

In another trial, Fomentini et al., (2015), yield used three different dietary treatments: MOS, avilamycin and halquinol. The trial used these prebiotics in combination and alone within the treatments that were offered. They observed that broilers that were fed without growth promoters demonstrated a lower BW when compared to those fed antimicrobials and MOS. The inclusion of MOS or antimicrobials alone or in combination, improved FCR until 49 d-of-age when compared to the control group. No differences were observed ($p>0.05$) in carcass yield or prime cuts, or final weight associated with the inclusion of MOS or antibacterial in the broiler's diet.

Table III. Effects of dietary treatment on the broiler on the live body weight, carcass weight and yield.

Variable	**Dietary Treatments**					
	CON	**MOS**	**BAC**	**M + B**	**SEM**	**P - value**
Live Body Weight, lb	6.08	6.02	6.11	6.14	0.11	NS
Carcass Weight, lb	4.37	4.42	4.40	4.40	0.09	NS
Carcass yield, %	71.93	72.69	72.48	71.93	0.45	NS

The four dietary treatments consisted of CON = Commercial Control (CON), MOS = Control + MOS (0.1% rate of inclusion), BAC = Control + BAC (0.05% rate of inclusion), and M + B = Control + MOS + BAC.

The increased FCR found at the 49 d-of-age due to the diet treatments, demonstrated that adjustments in the inclusion level, are necessary in order to elevate muscle deposition and reach an improved carcass weight (Fomentini et al., 2015).

Table IV. RT-PCR detection for the presence *of Campylobacter spp.* in broilers.

Treatment	Positive	Negative	Total
CON	4	11	15
MOS	4	11	15
BAC	4	11	15
M+B	0	15	15
Total	12	48	60

Statistics	Value	P-Value
Chi-square test of independence	5.00	0.1718

The four dietary treatments consisted of CON = Commercial Control (CON), MOS = Control + MOS (0.1% rate of inclusion), BAC = Control + BAC (0.05% rate of inclusion), and M + B = Control + MOS + BAC.

A chi – squared test of independence was performed for the RT-PCR. Each treatment detection be that Positive or Negative for *Campylobacter*, was treated as a frequency. The results demonstrated that the chi-squared p-value was greater than 0.05 ($P>0.05$). These results mean that the null hypothesis is accepted and the observed results are independent of the treatments. The positive or negative detection of *Campylobacter* is independent from the treatments that were performed, even if the results for M + B were effective in by resulting in none of the broilers being affected by *Campylobacter*. Statistically these results do not demonstrate in detail the significance of the *Campylobacter* absence in M + B, due to unknown reasons. Further investigation should be performed in order to ascertain the value of the data outside of statistical analysis.

This RT-PCR was also performed on fecal samples for *Salmonella* detection, which resulted that all pens did not contain any type of *Salmonella* strain.

Pedroso et al., (2013), ran a trial in which they evaluated if withdrawing antibiotics or including probiotics and prebiotics in diets, would decrease the antibiotic resistant genes in pathogens. To prove this, the trial ran a PCR in which determined the presence or absence of the pathogen in poultry litter. Observations ranged as the bacterial diversity that was found in the broilers that were fed prebiotics or probiotics. Pathogens such as *Staphylococcus aureus* and *Clostridium perfringens* were among the pathogens that were found. The trial ran a 16s rRNA detection method, and after various litter sample all the flocks were positive for *Salmonella*, except the group that was treated with the probiotic Primalac. The group that had a prebiotic and probiotic combination of All-Lac + BioMos although resulted in positive for *Salmonella*, the percentage of its presence was lower than the rest of its counterparts. These findings prove that not all probiotics and prebiotics are capable of preventing the pathogens colonization in the broiler's gastrointestinal tract but, specific ones are capable to prevent and even reduce the population of specific pathogens, such as *Salmonella*.

Another trial performed by Rudi et al., (2004), used Direct Real-Time PCR (RT-PCR) for the quantification and detection of *C. jejuni* in broiler fecal samples. Although the objective of this trial was to develop an assay for *C. jejuni* from cecal content, this trial obtained samples from thirty on different flocks and ran the RT-PCR for all of them. The results showed that nineteen of the flocks resulted positive for *C. jejuni* while the rest were negative. Among the observations found in this trial was the appearance of different *C. jejuni* strains, also most of the *C. jejuni* content was found in the cecal samples rather than the fecal samples. Among the main organs of poultry where bacterial colonization can occur, the poultry cecum is most susceptible organ to a *C. jejuni* colonization (Knudsen et al., 2006). The problem with how to asses these pathogens is that many of the colonization methods used by *C. jejuni* are not completely understood and many of them

are still under investigation. The detection of *C. jejuni* is of vital importance, many birds do not demonstrate symptoms of infection until a post-mortem examination determines the cause of death is by *C. jejuni* infection. Probiotics and prebiotics, are most effective in specific strains and levels of inclusion, in this trial only M + B was capable of receiving no infected pens. However, statistically the results are independent of the treatment, additionally MOS and BAC independently had positive *C. jejuni* pens, so further investigation for specific strains and inclusion levels for probiotics and prebiotics to combat *C. jejuni* are necessary.

6 CONCLUSION

In conclusion, this trial has demonstrated that additives such as prebiotics and probiotics are not a straightforward additive to use on broiler feed. Throughout the trial while the additives did not show any signs of adverse effects on the flock, it did not show any beneficial traits either. Further investigation should be done in order to ascertain the quantity and species of beneficial bacteria to use as additives. Although prebiotics and probiotics did not have any adverse effects on the flock, to use them effectively can cause beneficial effects on the broiler performance. Additional effects may and are not limited to improve the gastrointestinal tract from pathogens that may cause huge losses in the production, such as *Salmonella* and *Campylobacter*.

7 RECOMMENDATIONS

According to this study and previous research, discrepancies on the use of probiotics and prebiotics is very common. Future investigations should be focused on the accuracy of the use of these additives on different species of animals. Obtaining a more accurate study protocol on the use of additives will be able to assess the threat that foodborne pathogens present on production animals. Inclusion levels, prebiotic and probiotic specific species and the quantity use of these additives, among many other factors, should be analyzed and tested, to be able to improve performance and carcass yield.

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